### Acute upper airway responses to hypoglossal nerve stimulation during sleep in obstructive sleep apnea

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Acute upper airway responses to hypoglossal nerve stimulation during sleep in obstructive sleep apnea

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At a glance commentary:

Scientific Knowledge on the Subject: Hypoglossal nerve stimulation is a potential novel therapeutic approach for patients with obstructive sleep apnea, although its ability to relieve pharyngeal airflow obstruction has not been determined.

What This Study Adds to the Field: Hypoglossal nerve stimulation produced marked dose-related increases in airflow without arousing patients from sleep, suggesting potential therapeutic efficacy across a broad range of sleep apnea severity.

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Abstract

Introduction: Hypoglossal nerve stimulation (HGNS) recruits lingual muscles, reduces pharyngeal collapsibility and treats sleep apnea. We hypothesized that graded increases in HGNS relieve pharyngeal obstruction progressively during sleep.

Methods: Responses were examined in thirty patients with sleep apnea who were implanted with an HGNS® system. Current (mA) was increased stepwise during non-REM sleep. Frequency and pulse width were fixed. At each current level, stimulation was applied on alternating breaths, and responses in maximal inspiratory airflow ($V_{\text{max}}$) and inspiratory airflow limitation (IFL) were assessed. Pharyngeal responses to HGNS were characterized by the (a) current levels at which $V_{\text{max}}$ first increased and peaked (flow capture and peak flow thresholds), and the (b) $V_{\text{max}}$ increase from flow capture to peak ($\Delta V_{\text{max}}$).

Results: HGNS produced linear increases in $V_{\text{max}}$ from unstimulated levels at flow capture to peak flow thresholds (215±21 to 509±37mL/s, mean±SE, p<0.001) with increasing current from 1.05±0.09 to 1.46±0.11mA. $V_{\text{max}}$ increased in all patients and IFL was abolished in 57% of patients (non-IFL subgroup). In the non-IFL compared to IFL subgroup, the flow response slope was greater (1241±199 vs. 674±166mL/s/mA, p<0.05) and the stimulation amplitude at peak flow was lower (1.23±0.10 vs. 1.80±0.20mA, p<0.05) without differences in peak flow.

Conclusions: HGNS produced marked dose-related increases in airflow without arousing patients from sleep. Increases in airflow were of sufficient magnitude to eliminate IFL in the majority of patients and both IFL and non-IFL subgroups achieved normal or near-normal levels of flow, suggesting potential HGNS efficacy across a broad range of sleep apnea severity.

Word count: 250
Introduction

Obstructive sleep apnea is characterized by recurrent episodes of upper airway obstruction during sleep. Airflow obstruction is thought to result from decreases in pharyngeal neuromuscular activity at sleep onset. These episodes lead to intermittent hypoxemia and recurrent arousals from sleep, accounting for the long-term neurocognitive, metabolic and cardiovascular sequelae of this disorder. Nasal continuous positive airway pressure (CPAP) remains the mainstay of treatment for moderate to severe obstructive sleep apnea. Difficulties adhering to therapy can limit its effectiveness in the home setting, and alternatives have been generally less effective in relieving upper airway obstruction during sleep.

Hypoglossal nerve stimulation (HGNS) has been piloted as treatment for obstructive sleep apnea. Implantable HGNS systems have been developed to stimulate the hypoglossal nerve during inspiration, and recruit the lingual musculature, leading to decreases in pharyngeal collapsibility during sleep. Motor activity protrudes the tongue, and mitigates airflow obstruction during sleep. Resulting increases in inspiratory airflow can account for observed reductions in sleep apnea severity, although residual airflow obstruction can persist in some patients. Nevertheless, airflow responses to graded increases in HGNS have not been described; nor has the magnitude of these responses been well characterized.

The primary purpose of the present study was to characterize airflow responses to HGNS in patients implanted with a novel HGNS system. We hypothesized that (1) graded increases in HGNS intensity would result in progressive relief of upper airway obstruction (improvements in maximal inspiratory airflow), and that (2) these responses are of sufficient magnitude to abolish airflow obstruction without arousing patients from sleep. Our findings have major implications for HGNS titration and predicting...
responses to HGNS therapy. Some of the results in the present study have been previously reported in abstract form.21,22

Methods

Patient Population

Thirty obstructive sleep apnea patients who were implanted with a novel HGNS system (HGNS® Apnex Medical, Inc.) were recruited. Written informed consent was obtained. Key eligibility criteria for implantation were (a) an apnea-hypopnea index of >20 episodes per hour (predominantly obstructive hypopneas) (b) and a central apnea index of ≤5% on a screening sleep study.

Experimental Techniques

Baseline Sleep Study: Each patient underwent a standard overnight sleep study to characterize sleep and breathing patterns. Airflow was assessed with a nasal pressure cannula and an oronasal thermistor. Hypopneas were defined by a >50% in airflow amplitude, or a discernable reduction in airflow that was associated with either a >4% oxyhemoglobin desaturation or an arousal from sleep. Apneas were defined by a >90% reduction in airflow for ≥10 seconds.

HGNS Device and Implantation Procedure: Patients were implanted with a stimulating lead utilizing a guarded bipolar electrode array within an insulating cuff to prevent current spread and focus stimulation on the nerve itself (see Online Methods for details). The neurostimulator, respiration sensing and stimulation leads were surgically implanted under general anesthesia. Briefly, the cuff of the stimulating lead was placed on the hypoglossal nerve distal to branches innervating the styloglossus and hyoglossus muscles, and placement was verified intraoperatively with fluoroscopic assessment of pharyngeal opening during stimulation. The stimulation lead body was connected to the neurostimulator, which was implanted in the ipsilateral infraclavicular space subcutaneously. Two
respiratory impedance sensing leads were tunneled subcutaneously toward the midline and then bilaterally along each costal margin. Adverse events were related to device implantation and are described in the online Methods (Table E1).

**Awake Titration Study:** Approximately one month after HGNS implantation, the twitch and tongue movement thresholds were determined by the lowest current level at which lingual muscle activation and bulk movement occurred, respectively.

**Titration Sleep Study:** Each patient then returned for another overnight sleep study to determine the effect of stimulation intensity (current) on tidal airflow during sleep (see below). A mask and pneumotachograph (n=26) or nasal cannula (n=4) was used to quantify airflow responses to stimulation.

**Experimental Protocol**

The HGNS system was designed to stimulate during inspiration. In this titration protocol, alternating breaths were stimulated so that responses in inspiratory airflow could be compared to adjacent unstimulated breaths during sleep. HGNS was applied with increasing current amplitudes from 0 to 4 mA while frequency and pulse width was fixed at 40 Hz and either 90 µs (n=26) or 60 µs (n=4). Flow responses in patients stimulated with 40 Hz and 60 µs did not differ, leading us to combine results from all patients.

During sleep, stimulation current was titrated upward in 0.1 to 0.3 mA steps until the airflow response plateaued or the patient aroused. Arousal was defined by a visible shift in EEG rhythm, increase in heart rate, or increase in maximal inspiratory airflow from baseline levels at stimulation offset.

**Data Analysis**

Maximal inspiratory airflow (V_{max}) was measured during stimulated and adjacent unstimulated breaths during stable NREM sleep, as defined by stability in V_{max} immediately before and after stimulated
breaths. At each stimulation level, breaths were assessed for the presence or absence of inspiratory flow limitation (IFL or non-IFL). Airflow responses at increasing current were characterized by: (1) the flow capture threshold at which airflow began to increase, (2) the peak flow threshold at which V_max or peaked or plateaued with or without the elimination of IFL. The stimulus response slope was calculated as the quotient of differences in V_max and current between the peak and capture flow thresholds. Unstimulated baseline levels of airflow were measured to assess for stability in the state of pharyngeal patency during sleep over the range of current applied.

Statistical Analysis

Student t-tests were used to compare airflow on and off stimulation (peak vs. baseline), and least squares linear regression was utilized to characterize airflow responses to graded levels of stimulation. The Pearson product moment correlation coefficient was calculated to examine the association between baseline and peak flow across the entire group. Least squares linear regression was also used to assess for drift in unstimulated airflow levels as current was varied. The sensitivity of the flow response to stimulation current was examined in 25 patients in whom flow capture thresholds was determined. Groups were stratified by the presence or absence of IFL at the peak flow threshold in order to compare flow responses. Results were expressed at means ± SEM, except in Table 1 where values are represented as mean ± SD. Statistical significance was inferred at a p<0.05 level.
Results

Patient Characteristics

The patients’ demographic and anthropometric characteristics and baseline sleep study results are described in Table 1. The patients were middle aged and moderately obese men and women with moderate and severe obstructive sleep apnea. By design, these patients had predominantly obstructive hypopneas rather than apneas. Sleep architecture was characterized by elevated N1 and reduced REM sleep.

Single breath stimulation airflow responses

Stimulation responses are illustrated for one representative patient in NREM sleep at three stimulation current levels (Figure 1). In each panel, two stimulated breaths are shown (see stimulation marker signal at bottom and stimulus artifact in submental electromyogram (EMG<sub>SM</sub>), and are bracketed by adjacent unstimulated breaths during stable non-REM sleep. Unstimulated breaths displayed evidence of severe inspiratory airflow limitation as characterized by an early plateau in inspiratory flow at a low level and high frequency mid-inspiratory oscillations in airflow (consistent with snoring). During unstimulated breaths, maximal inspiratory airflow remained stable, regardless of the stimulation amplitude applied on the stimulated breaths. The return of flow to the baseline levels on the intervening unstimulated breaths provided evidence that stimulation was not causing arousal from sleep. In contrast, a graded response in maximal inspiratory airflow (downward direction) was observed with increasing levels of maximal inspiratory airflow as current was increased. Inspiratory airflow limitation persisted at low (Left panel) and mid-levels (Middle panel) of stimulation, but was abolished at still higher current level applied (Right panel). When a low level of current was applied (1.7 mA, Figure 1, left panel), maximal inspiratory airflow (V<sub>max</sub>) did not increase relative to adjacent breaths before and after stimulation,
indicating that the applied current remained below the flow capture threshold. As current was increased to 2.0 mA (middle panel) maximal inspiratory airflow \( (V_{\text{I}}\text{max}) \) increased during the stimulated compared to unstimulated breaths. Nevertheless, inspiration remained flow-limited, as evidenced by an early peak in inspiratory airflow followed by a roll-off and plateauing of inspiratory flow later in inspiration (indicative of ‘negative effort dependence’, a recognized phenomenon in collapsible biologic conduits \(^{25,26}\). When the stimulus intensity was increased to 2.5 mA, \( V_{\text{I}}\text{max} \) increased further, and inspiratory airflow no longer plateaued, indicating the flow limitation had been abolished. Of note, stimulation was not associated with any shift in EEG frequency, change in heart rate or increase in maximal inspiratory airflow during unstimulated breaths, indicating that arousal had not occurred.

The flow responses observed in the representative patient in Figure 1 were utilized to generate an illustrative flow response curve in Figure 2. As increasing stimulation was applied, \( V_{\text{I}}\text{max} \) increased linearly from the flow capture threshold to the peak flow threshold, but inspiratory airflow remained flow limited over this current range. Once current exceeded the peak flow threshold, increases in stimulation amplitude no longer generated any further increases in inspiratory airflow and inspiratory flow limitation was abolished.

**Flow Responses Characteristics**

*Airflow response to stimulation:* Maximal airflow responses to stimulation are illustrated for the entire group in Figure 3 (n=30). During NREM sleep, patients exhibited a mean \( V_{\text{I}}\text{max} \text{ off} \) stimulation of 215±21 mL/s and \( \text{on} \) stimulation of 509±37 mL/s, making for a mean increase in \( V_{\text{I}}\text{max} \text{ off} \) of 294±33 mL/s at the peak flow threshold. \( V_{\text{I}}\text{max} \) increased in all 30 patients \( \text{on} \) compared to \( \text{off} \) stimulation, and the level of stimulated peak flow correlated with the unstimulated flow \( (r=0.50, p=0.005) \), suggesting that the degree of airway opening depended on the severity of upper airway obstruction at baseline.

Moreover, inspiratory airflow limitation was abolished altogether in 17 patients (Figure 3, see open
circles, Stimulation ON) and improved markedly in the remaining 13 patients (Figure 3, see closed circles, Stimulation ON). A similar increase in airflow was achieved in both the IFL and non-IFL groups (256±31 v 323±52, p=0.15), indicating substantial improvements in pharyngeal patency during stimulation in both groups. These groups did not differ in unstimulated (241±25 vs. 182±36 mL/s) or peak flow levels (564±58 vs. 438±35 mL/s). Nevertheless, the IFL subgroup required greater current to achieve peak flow (1.80±0.20 v 1.23±0.10mA, p<0.05).

**Sensitivity of the flow response to stimulation:** Stimulation generated progressive increases in airflow from a flow capture threshold (216±24) mL/s at 1.05±0.09 mA to a peak flow threshold of 538±41 mL/s at 1.46±0.11 mA. This 0.41±0.06 mA (p<0.001) increase in stimulation current was associated with increases in airflow of 321±36 mL/s (p<0.001), indicating marked sensitivity in the flow response to stimulation.

Further insight into stimulus response mechanisms can be gained by comparing the sensitivity of the flow response in groups with and without flow limitation from the flow capture to peak flow thresholds (Figure 4). These groups did not differ significantly in age, BMI or sex; nor did the flow limited group differ significantly from the non-flow limited group in the baseline unstimulated level of flow (174±43 vs. 244±28 mL/s), the flow capture threshold (1.23±0.18 vs. 0.93±0.10 mA), or in the twitch motor threshold level (0.78±0.12 vs. 0.67±0.04 mA), respectively. Compared to the flow limited group (closed circles), the flow response in the non-flow limited group (open circles) was greater (see steeper slope, 674±166 vs. 1241±199 mL/s/ mA, p<0.05), indicating greater sensitivity in the response to stimulation in this group. Peak airflow did not differ in the flow limited compared to non-flow limited group (438±35 vs. 564±58 mL/s), although the flow limited group required a greater increase in stimulation current to achieve peak flow from the flow capture threshold (0.57±0.12 vs. 0.30±0.03 mA, p<0.05). Of note, both groups attained normal or near normal levels of maximal inspiratory airflow during sleep of ~400 mL/s or greater (see shaded region).
Discussion

Acute unilateral stimulation of the hypoglossal nerve during sleep in patients with obstructive sleep apnea resulted in progressive increases in inspiratory airflow with increasing stimulation intensity. Stimulation increased airflow markedly and abolished inspiratory flow limitation in the majority of patients. Moreover, inspiratory airflow returned to baseline unstimulated levels during and immediately following the stimulated breath, suggesting that HGNS exerted a direct effect on lingual muscles and airway patency without arousing patients from sleep. Airflow increased in all patients, and rose progressively with stimulus amplitude. Such consistent, progressive flow responses suggest a direct relationship of tongue position to pharyngeal patency during sleep. Finally, the increases in airflow were of sufficient magnitude to suggest potential therapeutic efficacy of HGNS across a broad range of sleep apnea severity.

Mechanism for increased airflow during stimulation

In previous studies, investigators have demonstrated that electrical stimulation of the genioglossus increases upper airway flow during sleep in sleep apnea patients. These increases have been attributed to decreases in pharyngeal collapsibility (critical pressure, \( \text{P}_{\text{crit}} \)) and the back pressure to inspiratory airflow. In previous studies, stimulating the genioglossus muscle and hypoglossal nerve led to an approximately 3 to 5 cm H\(_2\)O decrease in \( \text{P}_{\text{crit}} \), which can account for an approximately 150 to 250 mL/s increase in maximal inspiratory airflow. In the present study we extend these observations by characterizing flow responses over a range of stimulus amplitudes and demonstrate even greater increases in airflow approximating 300 mL/s, possibly owing to differences in cuff placement, nerve anatomy, stimulation intensity or increased sensitivity of the flow response. Peak flow responses were likely underestimated since inspiratory airflow limitation was eliminated in the
majority of patients and maximal inspiratory airflow can no longer increase once flow limitation resolves. These findings suggest that the current HGNS approach can produce substantial relief of upper airway obstruction during sleep.

Flow response curve characteristics

We characterized responses in airway patency by delineating flow increases from baseline over a range of current amplitude. In analyzing flow response curve characteristics, we found that the slope of the flow response to stimulation was greater in the patients whose airflow obstruction (inspiratory flow limitation) abated than those whose IFL persisted. Differences in the slope of the flow response curve could be related to variability in the degree of neuromechanical coupling or cuff placement between the flow-limited and non-flow limited groups. These slope differences were not associated with differences in the flow capture threshold during sleep or twitch threshold during wakefulness, suggesting that cuff placement and nerve impedance were similar between groups. Rather, peak flow was achieved at lower stimulation amplitudes in the non-flow limited group, suggesting enhanced mechanical effects of lingual muscle contraction on the pharynx. This effect could reflect greater linkage between the tongue and other pharyngeal structures, owing to differences in lingual muscle fiber orientation, lingual-palatine linkage and/or pharyngeal site of collapse. Alternatively, lingual muscle recruitment patterns could differ among patients, as suggested by observed decreases in inspiratory airflow in one patient at stimulation amplitudes well above the peak flow threshold, which were also associated with tongue retractor during wakefulness and sedation. These decreases suggest current spread to lingual retractor muscles, which could have resulted from a more proximal cuff electrode placement. Finally, the enhanced flow peak flow response correlated with baseline unstimulated flow, suggesting that baseline differences in the severity of airflow obstruction can also account for observed differences in peak flow responses to a given stimulus amplitude. Thus, augmented flow responses may result from increased mechanical linkage between lingual and pharyngeal structures, a predominance of lingual protrusor
muscle recruitment and/or lesser degrees of airflow obstruction at baseline.

Arousals

HGNS evolved from initial studies examining the effects of transcutaneous submental stimulation on upper airway patency. Investigators demonstrated improvements in airway patency that were later thought likely related to arousals rather than selective stimulation of the lingual muscles during sleep. Subsequently, investigators documented arousal thresholds during submental stimulation, which confounded assessment of airflow responses and limited clinical applicability of this technique during sleep. Investigators further refined the stimulation technique by inserting temporary fine wire electrodes into lingual muscles, and demonstrated that protrusor muscle stimulation mitigated and retractor muscle stimulation worsened pharyngeal patency during sleep. In these prior studies, investigators scrutinized EEG and ECG signals to exclude responses associated with cortical or autonomic activation. Additional studies with implantable nerve cuff and fine wire electrodes have demonstrated responses to selective lingual muscle stimulation during sleep and anesthesia. In the present study, we also screened for evidence of cortical and autonomic activation, and further required strict temporal synchrony between the stimulus burst and airflow response to exclude arousal responses from our analysis. The following provide further evidence that arousal did not confound our assessment of flow responses during sleep. First, flow returned to baseline levels on alternating unstimulated breaths as shown in Figure 1. Second, the graded, linear response in flow to increasing stimulation intensity up to the peak flow threshold as shown in Figure 2 is consistent with a dose-dependent mechanical opening of the airway rather than an arousal mechanism. Third, the standard protocol of the sleep titration study was to increase stimulation intensity until arousals were actually observed to occur. Fourth, flow limitation persisted in selected patients over a broad range of stimulation currents, which could only occur during sleep rather than wakefulness. These analyses reinforce our conclusion that unilateral HGNS produces progressive relief of upper airway obstruction.
without arousing patients from sleep.

Limitations

Several limitations should be considered in interpreting our findings. First, our protocol stimulated every other breath in order to establish a quasi-steady state baseline level of airflow obstruction. Unstimulated airflow levels in our apneic patients were often insufficient to prevent recurrent apneas or hypopneas, and breathing patterns could not be completely stabilized until higher levels of HGNS were applied, when flow and tidal volumes increased. While airflow and ventilation increased progressively, unstimulated flows remained constant on adjacent breaths, and provided a stable baseline from which to gauge airflow responses across stimulation current levels. Second, esophageal manometry was not used to monitor inspiratory effort and assess for the presence of inspiratory flow limitation. We could nevertheless rely on the inspiratory flow contour to distinguish flow limited from non-flow limited breaths, since the inspiratory plateau remains a well validated index of inspiratory airflow limitation during natural sleep. Third, we recognized that arousal could confound our assessment of flow responses and designed our experimental protocol and analytic approaches to minimize this possibility, as described above. Fourth, our study assessed acute airflow responses to HGNS, and did not address factors associated with the chronic use of HGNS therapy. Fifth, flow response curves were not delineated in all body positions and sleep stages due to time constraints and titration protocol development. Nevertheless, our streamlined methods allowed us to enlarge our patient sample across sites, thereby increasing the generalizability of our findings.

Implications for therapy

Our findings have several implications for HGNS therapy. First, the airflow response to peak stimulation was of sufficient magnitude to relieve upper airway obstruction during sleep and reduce sleep apnea severity. In patients with baseline levels of maximal inspiratory airflow during sleep disordered
breathing episodes of 50 to 250 mL/s, a mean increase of 294 mL/s would likely yield relatively normal levels of peak inspiratory airflow found in asymptomatic snoring and normal non-snoring individuals during sleep \(^{37;38}\). Second, flow changed instantaneously with stimulation and increased progressively with stimulus intensity, suggesting that increases in flow are a direct effect of stimulation rather than a result of arousals from sleep. Third, inspiratory airflow was exquisitely sensitive to small changes in stimulus amplitude, suggesting that peak flow responses, as discerned from the flow response curve, should be targeted for HGNS therapy and that further increases in stimulation intensity will not yield further benefit. Fourth, a brisk flow response was associated with complete elimination of upper airway obstruction in the majority of patients. The stimulus intensity required to generate normal levels of airflow was greater in those with persistent flow limitation, implying that other pharyngeal and/or lingual muscles must still be recruited to completely abolish inspiratory flow limitation during sleep. Further studies in additional patients, sleep stages and body positions will be required to determine the clinical and physiologic predictors of this response.
Table 1

Demographic, anthropometric and baseline sleep study characteristics

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ODI 4%, oxyhemoglobin desaturation index including events with > 4% desaturation; TST, total sleep time; N1, stage 1 non-REM sleep; N2, stage 2 non-REM sleep; N3, stage 3 non-REM sleep; REM, rapid
eye movement sleep.
**Figure Legends**

**Figure 1.** Representative polysomnographic recording examples of HGNS response at low (1.7 mA, Left panel), moderate (2.0 mA, Middle panel) and high (2.5 mA, Right panel) levels of stimulation in one patient. In each panel, two stimulated breaths are shown (see stimulation marker signal at bottom and stimulus artifact in submental electromyogram (EMG<sub>SM</sub>), and are bracketed by adjacent unstimulated breaths during stable non-REM sleep. Unstimulated breaths displayed evidence of severe inspiratory airflow limitation as characterized by an early plateau in inspiratory flow at a low level and high frequency mid-inspiratory oscillations in airflow, consistent with snoring. During unstimulated breaths, maximal inspiratory airflow did not change across all stimulation levels, indicating that severe inspiratory flow limitation persisted across stimulation levels. In contrast, a graded response in maximal inspiratory airflow (downward direction) was observed with increasing levels of maximal inspiratory airflow as current was increased. Inspiratory airflow limitation persisted at low (Left panel) and mid-levels (Middle panel) of stimulation, but was abolished at the highest stimulation level applied (Right panel). Please note time lags of respiratory impedance signal (HGNS (Z)) and stimulus current marker signal (STIM) of ~400 ms and ~250 ms, respectively, relative to the airflow and abdominal piezo-electric belt (ABD) signals due to signal processing and transmission from the implanted neurostimulation device. L. EOG, left electrooculogram; R. EOG, right electrooculogram; F4M1, C4M1, and O2M1 electroencephalogram leads; EMG<sub>SM</sub>, submental electromyogram; ECG, electrocardiogram; SaO<sub>2</sub>, oxyhemoglobin saturation; flow, tidal airflow; ABD, abdominal piezo-electric gauge; HGNS (Z), implanted respiratory impedance sensor; Stim, stimulation current marker signal.

**Figure 2.** Inspiratory airflow (V<sub>max</sub>) response to increasing HGNS current amplitude during NREM sleep for stimulated and unstimulated breaths in the patient illustrated in Figure 1. As stimulation current increased beyond the **Flow Capture Threshold**, V<sub>max</sub> increased linearly until the **Peak Flow Threshold** was
attained, at which point $V_{\text{I, max}}$ plateaued as increasing stimulus current was applied. Please note that inspiratory flow limitation persisted at intermediate current levels (closed circles). Further increases in current abolished inspiratory flow limitation (open circles).

**Figure 3.** Maximal inspiratory airflow ($V_{\text{I, max}}$) with stimulation OFF (mean baseline unstimulated breaths) and ON (at peak flow threshold) is represented for each patient and for the group as a whole (see means ± SEM). A significant increase in $V_{\text{I, max}}$ was observed for the group as a whole ($p<0.001$). At the peak flow threshold, flow limitation was eliminated in 17 of 30 patients (see open circles, Stimulation ON), and persisted in the remaining 13 patients (see closed circles, Stimulation ON).

**Figure 4.** Maximal inspiratory airflow ($V_{\text{I, max}}$) vs. Stimulation Current (mA, milliamperes) in groups with (closed circles) and without (open circles) inspiratory flow limitation at the Peak Flow Threshold. The flow response slope in the non-flow limited group was greater than that in the flow limited subgroup ($1241±199$ vs. $674±167$ mL/s/mA, $n=25$, $p<0.05$). Lower levels of stimulation current were required to achieve peak airflow in the non-flow limited compared to flow-limited subgroup ($1.23±0.10$ vs. $1.80+/-.020$ mA, $n=25$, $p<0.05$), although peak inspiratory airflow did not differ between non-flow limited and flow limited subgroups ($564 ± 58$ vs. $438 ± 35$ mL/s). Both groups attained normal or near normal levels during sleep of $~400$ mL/s or greater (see shaded region).
Figure 1: Representative recordings in one patient at low, moderate and high levels of stimulation

L.EOG (50μV)  
R.EOG (50μV)  
F4M1 (50μV)  
C4M1 (50μV)  
02M1 (50μV)  
EMG EMG (10μV)  
ECG  
SaO2 (%)  

FLOW (500mL/h)  

ABD  
HGNS (Ω)  
STIM (mA)  
1.7 mA  
2.0 mA  
2.5 mA  

2 s
Figure 2: Flow response curve in a representative patient
Figure 3: Baseline (unstimulated) and peak (stimulated) maximal inspiratory airflow ($V_{\text{Imax}}$) during NREM sleep.
Figure 4: Maximal inspiratory airflow ($V_{\text{I}}$ max) vs. Stimulation Current (mA, milliamperes) in groups with and without inspiratory flow limitation at the peak flow threshold.

- Flow Limited
- Non-Flow Limited

Peak Flow Thresholds
Flow Capture Thresholds

* $p<0.05$
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Acute upper airway responses to hypoglossal nerve stimulation during sleep in obstructive sleep apnea

On Line Data Supplement/Methods

Alan R. Schwartz, Maree Barnes, David Hillman, Atul Malhotra, Eric Kezirian, Philip L. Smith, Thomas Hoegh, Daniel Parrish, Peter R. Eastwood
Methods

Patient Population

Thirty patients with moderate to severe obstructive sleep apnea were recruited as part of a multi-center clinical trial and implanted with a novel HGNS system (HGNS® Apnex Medical, Inc.), as previously described. Written informed consent for this study was obtained for the protocol, which was approved by the local institutional review boards. Key eligibility criteria for implantation were (a) an apnea-hypopnea index of greater than 20 episodes per hour (predominantly obstructive hypopneas) (b) and a central apnea index of no greater than 5% on a screening overnight sleep study. Patients with concomitant medical illnesses were also excluded. Of these, 30 patients underwent HGNS titration studies consisting of quantitative measures of airflow over a range of HGNS stimulation intensity during sleep, and provide the patient sample herein.

Experimental Techniques

*Baseline Sleep Study*: Each patient underwent a baseline overnight sleep study to characterize sleep and breathing patterns. Standard polysomnographic techniques were utilized including surface electroencephalograph (EEG) leads (F3-M2, F4-M1, C3-M2, C4-M1, O1-M2, O2-M1), submental electromyogram (EMG), left and right electrooculograms (L. and R. EOG) to stage sleep. A single modified electrocardiogram (ECG) II Lead placement was employed to monitor cardiac rhythm. Respiratory monitoring included oronasal airflow with nasal pressure cannula and thermistor, oxyhemoglobin saturation (SaO2), and the thoraco-abdominal effort gauges (respiratory impedance monitors). Hypopneas were defined by a >50% in airflow amplitude in the nasal pressure cannula signal during sleep, or a discernable reduction in airflow that was associated with either a ≥4% oxyhemoglobin
desaturation or an arousal from sleep (a 3s shift in EEG frequency). Apneas were defined by a >90%
reduction in airflow from baseline in both the oronasal thermistor and nasal cannula signal for at least
10 seconds.

**HGNS Device and Implantation Procedure:** Patients were implanted with a bipolar stimulating lead. A
guarded bipolar electrode array was mounted within an insulating cuff. The lead included three
electrode contacts placed longitudinally along the nerve body. The polarity of the center electrode was
opposite that of two flanking electrodes, and served to focus the stimulation current on the nerve itself
with stimulating other nearby muscles or nerves.

The neurostimulator, respiration sensing and stimulation leads were surgically implanted under general
anesthesia. Briefly, an incision was made below and parallel to the inferior border of the right mandible.
The main right hypoglossal nerve trunk was exposed below the submandibular gland and superior to the
digastric tendon, distal to branches innervating the styloglossus and hyoglossus muscles. The cuff of the
stimulating lead was placed on the hypoglossal nerve and correct cuff placement was verified
intraoperatively with fluoroscopic assessment of pharyngeal opening during brief stimulation. The
stimulation lead body was then tunneled below the platysma through the neck to the neurostimulator,
which was implanted in the ipsilateral infraclavicular space subcutaneously. Two respiratory impedance
sensing leads were tunneled subcutaneously toward the midline and then bilaterally along each costal
margin. Following surgery, a healing period of approximately 30 days was allowed without stimulation.
Adverse events were related to the implantation procedure and are described in Table E1. Serious
events requiring surgical intervention included cuff dislodgement (n=2) and hematoma/infection (n=1).
Other adverse events occurred transiently following the implantation procedure.

**Awake Titration Study:** Approximately one month after HGNS implantation, each patient returned to the
clinic for an awake titration study. During the awake titration, stimulation amplitude settings were
increased in a step-wise manner to determine the twitch and tongue movement thresholds at which lingual muscle activation and bulk movement were first observed, respectively.

**Titration Sleep Study:** Following the awake titration, each patient returned to the laboratory for another overnight sleep study to determine the effect of varying the stimulation intensity (current) on tidal airflow during sleep (see below). Sleep and breathing patterns were monitored as described above. In addition, a mask and pneumotachograph were used to monitor airflow and quantify airflow responses to stimulation (n=26) or nasal cannula in those who did not tolerate the mask and pneumotachograph (n=4). Patients monitored with a nasal cannula did not differ from those with pneumotachograph in age, body mass index or apnea-hypopnea index. A hypnotic was utilized to facilitate sleep monitoring as necessary.

**Experimental Protocol**

The HGNS stimulation system was designed to track the patient's respiratory pattern and stimulate during the inspiratory phase of each respiratory cycle. In contrast to stimulating each breath during standard therapy, this protocol was performed with a custom stimulation delivery mode which stimulated alternating breaths so that responses in inspiratory airflow could be compared to adjacent unstimulated breaths during sleep. HGNS was applied with increasing current amplitudes from 0 to 4 mA while frequency and pulse width was fixed at 40 Hz and 90 $\mu$s (n=26) or at 40 Hz and 60 $\mu$s (n=4), respectively. Flow responses in patients stimulated with 40 Hz and 60 $\mu$s did not differ from those in patients stimulated with 40 Hz and 90 $\mu$s, leading us to combine results from all patients in our analyses.

During sleep, stimulus current was titrated from the twitch threshold (n=25) or from bulk tongue movement (n=5) determined during awake titration to maximally tolerated levels (i.e., until patients could no longer reinitiate sleep). Stimulation current was incremented in approximately 0.1 to 0.3 mA.
steps until the airflow response to stimulation plateaued or the patient aroused from sleep (see below). Inspirations were stimulated repeatedly at each current level on alternating breaths whereas the standard therapy mode is to stimulate on every breath. Arousals were considered to have occurred if stimulation was accompanied by any shift in EEG rhythm either during or immediately after the stimulation burst, an increase in heart rate, or if maximal inspiratory airflow did not return to baseline levels at the offset of stimulation, as previously described.

Data Analysis

Airflow responses to HGNS were characterized during stable periods of NREM sleep as follows. Maximal inspiratory airflow (V_{I,max}) was measured during stimulated breaths and adjacent unstimulated breaths during stable NREM sleep, as previously described. At low levels of stimulation, airflow during stimulated breaths was insufficient to stabilize the breathing pattern and breaths were measured during obstructive apneas and hypopneas when stimulated breaths were bracketed by unstimulated breaths of similar V_{I,max} amplitude. Breaths were also assessed for the presence or absence of inspiratory flow limitation (IFL or non-IFL) at each stimulation level. In patients studied with nasal cannula (n=4) rather than pneumotachograph (n=26), flow was approximated by taking the root mean square transform of the nasal cannula signal. Flow signals from patients studied with nasal cannula (n=4) and uncalibrated pneumotachograph (n=5) were then scaled to the mean level of stable non-flow limited airflow in same sex patients studied with a calibrated pneumotachograph (n=21). Sensitivity analyses demonstrated no differences in baseline characteristics (demographic, anthropometric, sleep apnea severity indices) and current thresholds at capture and peak flow response thresholds (see below), leading us to combine data from uncalibrated and calibrated groups.

Airflow responses to increasing current were characterized as follows. The flow capture threshold was defined by the current level at which airflow increased during stimulation compared to adjacent
unstimulated breaths. The peak flow threshold was defined by the minimal current level associated with (a) the elimination of IFL (n=17), or when IFL persisted, (b) a peak in airflow (n=3) or a plateauing of $V_{max}$ with increasing current (n=10). Airflow at the peak flow threshold was taken to be the peak flow obtained during NREM sleep in response to stimulation. It is worth noting that marked decreases in airflow were observed in one patient at high stimulation amplitudes well above the peak flow threshold. Current levels at the flow capture and peak flow thresholds were used to define the stimulus response slope, which was taken to be a measure of the sensitivity of the response to stimulation. In addition, unstimulated baseline levels of airflow were measured to assess for stability in the state of pharyngeal patency during sleep over the range of current applied.

Statistical Analysis

Paired t-tests were used to compare airflow on and off stimulation (peak vs. baseline) and two-sample t-tests were used to compared demographic, anthropometric and flow response parameters between IFL and non-IFL subgroups. Least squares linear regression was utilized to characterize airflow responses to graded levels of stimulation and the drift in unstimulated levels of airflow across stimulation levels. The Pearson product moment correlation coefficient was calculated to examine the association between baseline and peak flow across the entire group. Least squares linear regression was also used to assess for drift in unstimulated baseline levels of airflow across current levels. The sensitivity of flow response to stimulation current was examined in 25 patients in whom stimulation was applied at sufficiently low amplitude to define the flow capture threshold. Groups were stratified by the presence or absence of IFL at the peak flow threshold. Demographic, anthropometric and flow response parameters were compared between subgroups with two sample student t-tests. Results were expressed at means ± SEM, except in Table 1 where values are represented as mean ± SD. Statistical significance was inferred at a p<0.05 level.
<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>% of all participants (n/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edema (swelling) of tissues or nerves</td>
<td>6.7% (2 / 30 )</td>
</tr>
<tr>
<td>Lip Weakness</td>
<td>6.7% (2 / 30 )</td>
</tr>
<tr>
<td>Abnormal scarring (keloid or hypertrophic)</td>
<td>3.3% (1 / 30 )</td>
</tr>
<tr>
<td>Change in salivary flow</td>
<td>3.3% (1 / 30 )</td>
</tr>
<tr>
<td>Transient hypoglossal nerve paresis</td>
<td>3.3% (1 / 30 )</td>
</tr>
<tr>
<td>Transient spinal accessory nerve paresis</td>
<td>3.3% (1 / 30 )</td>
</tr>
<tr>
<td>Tongue muscle fatigue, weakness or soreness</td>
<td>3.3% (1 / 30 )</td>
</tr>
<tr>
<td>Cuff dislodgement</td>
<td>6.7% (2 / 30 )</td>
</tr>
<tr>
<td>Hematoma /Infection (Explant required)</td>
<td>3.3% (1 / 30 )</td>
</tr>
</tbody>
</table>
Reference List


