Full Study Title

Hypoglossal Nerve Stimulation for Obstructive Sleep Apnea on Sympathetic and Vascular Function

Short Study Title

CARDIOSA-12

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Sponsors
American Heart Association
American Sleep Medicine Foundation

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1. **Eligibility Criteria:** All patients recruited into the study will have already been implanted with the Inspire® device. Additional inclusion criteria include:
   - As tolerating the therapeutic level during sleep can take time (weeks to months), all patients must be able to use the device at the therapeutic setting (> 20 hours per week for > 1 month) prior to enrollment based on compliance data.
   - The patients will be English-speaking and able to give written informed consent.

2. Exclusion Criteria

- Inspire® remote model 2500 or later is required. Patients with older remotes are not candidates due to limited adherence monitoring capabilities.
- Patients who have fallen asleep while driving resulting in accident or “near miss” accident within 1 year prior to HGNS implantation.
- Pregnant women will be excluded*
- Actively using positive airway pressure (PAP) therapy for treatment of OSA.
- Patients in whom the difference between sham and therapeutic voltages is within 0.1 V of 30% therapeutic voltage.

* Women of child bearing potential must NOT be pregnant or plan on becoming pregnant. This study involves temporarily stopping treatment of obstructive sleep apnea, which may harm the fetus. If applicable, the patient will need to take a urine pregnancy test after enrollment (prior to washout #1), and again prior to washout #2.

3. Study Objectives

Primary Outcome Measure:

- Change in mean 24-HOUR systolic ambulatory blood pressure values

  Aim 1: Determine the impact of hypoglossal nerve stimulation on 24-hour systolic ambulatory blood pressure in this cohort of patients with moderate-severe obstructive sleep apnea

  Hypothesis: The mean 24-hour systolic blood pressures will be improved at therapeutic levels of hypoglossal nerve stimulation and returned to pre-operative levels after 30-day sham period

Secondary Outcome Measures:

- Change in mean 24-HOUR diastolic ambulatory blood pressure values

  Aim 2a: Determine the impact of hypoglossal nerve stimulation on 24-hour diastolic ambulatory blood pressure in this cohort of patients with moderate-severe obstructive sleep apnea

  Hypothesis: The mean 24-hour diastolic blood pressures will be improved at therapeutic levels of hypoglossal nerve stimulation and returned to pre-operative levels after 30-day sham period

- Change in mean SLEEP systolic and diastolic ambulatory blood pressure values

  Aim 2b: Determine the impact of hypoglossal nerve stimulation on mean sleep systolic and diastolic blood pressures in this cohort of patients with moderate-severe obstructive sleep apnea

  Hypothesis: The mean sleep systolic and diastolic blood pressures will be improved at therapeutic levels of hypoglossal nerve stimulation and returned to pre-operative levels after 30-day sham period

- Change in Pre-Ejection Period (PEP) duration

  Aim 3: Determine the impact of hypoglossal nerve stimulation on pre-ejection period in this cohort of patients with moderate-severe obstructive sleep apnea

  Hypothesis: The pre-ejection period will be improved at therapeutic levels of hypoglossal nerve stimulation and returned to pre-operative levels after 30-day sham period

- Association of MSNA* and PEP with blood pressure measurements

  Aim 4: Determine if MSNA and PEP are positively associated with blood pressure measurements after adjusting for confounders of age, sex, body-mass index, baseline systolic BP, baseline diastolic BP, antihypertensive medication use.

  H1: There will be a significant association between alterations in systolic and diastolic blood pressure and muscle sympathetic nerve activity

  H2: There will be a significant association between alterations in systolic and

diastolic blood pressure and pre-ejection period duration

*Note: MSNA was part of Emory University protocol and will not be performed at the University of Pennsylvania.

- Change in Flow-mediated Dilation (FMD)

  Aim 4a: Determine the impact of hypoglossal nerve stimulation on flow mediated dilation in this cohort of patients with moderate-severe obstructive sleep apnea

  Hypothesis: The flow mediated dilation parameters will be improved at therapeutic levels of hypoglossal nerve stimulation and returned to pre-operative levels after 30-day sham period

- Change in Pulse Wave Velocity (PWV)

  Aim 4b: Determine the impact of hypoglossal nerve stimulation on pulse wave velocity in this cohort of patients with moderate-severe obstructive sleep apnea

  Hypothesis: The pulse wave velocity parameters will be improved at therapeutic levels of hypoglossal nerve stimulation and returned to pre-operative levels after 30-day sham period

- Change in Digit Symbol Substitution Test (DSST) score

  Aim 5: Determine the impact of hypoglossal nerve stimulation on digital symbol substitution test score in this cohort of patients with moderate-severe obstructive sleep apnea

  Hypothesis: The digital symbol substitution test score will be improved at therapeutic levels of hypoglossal nerve stimulation compared to after 30-day sham period

- Change in Psychomotor Vigilance Test (PVT) score

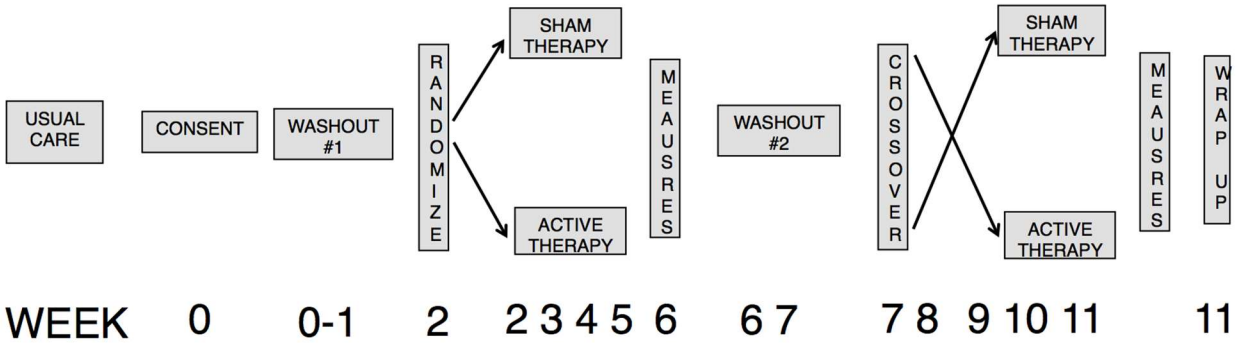
  Aim 6: Determine the impact of hypoglossal nerve stimulation on psychomotor vigilance test score in this cohort of patients with moderate-severe obstructive sleep apnea

  Hypothesis: The psychomotor vigilance test score will be improved at therapeutic levels of hypoglossal nerve stimulation compared to after 30-day sham period

4. Procedures and Data Collection

4A*. Pre-Study Procedures:* All patients in the cohort will have undergone implantation of HGNS for OSA at the University of Pennsylvania or Thomas Jefferson University as part of their ongoing care for their OSA. HGNS therapy is not experimental and is not under investigation per se for efficacy in this study. **Figure 3** shows the study flow. Patients will receive compensation for travel and time spent in the study ($100 per visit, plus $10 parking reimbursement for each cardiovascular measurement visit; total=up to $520). Reimbursement for parking may also be available. Compensation will be pro-rated, and will be in the form of mailed checks and/or ClinCards.

Figure 3: Study Flow



Note: We will leave +/- 14 days at each step to accommodate for scheduling issues, etc.

1. HGNS Device Implantation: Under general anesthesia, the HGNS device is implanted via 3 incisions: right neck (stimulation lead), right upper chest (pulse generator) and right lower chest (respiratory sensor). The incisions are connected via a tunneling device, integrating the system components. The pulse generator, using technology from cardiac pacemakers, utilizes the signal from the pressure sensor for timing of stimulation to the tongue nerve. Patients are monitored in post-anesthesia recovery until stable for discharge. The device remains "off" for 1 month to allow for adequate healing.

2. HGNS Device Activation (1-month post-op): As part of usual care, patients return to the Otorhinolaryngology clinic 1 month post-operatively to undergo activation of the implanted device. At this visit, a functional threshold (in volts) is obtained based on tongue motion during device stimulation. The patient is provided detailed instructions on self-adjustment of stimulation levels using the remote control. For the next month, the patient will have steadily increased stimulation strength by 0.1V every 2-3 nights within the preset range until the upper limit is reached. The patient has the full support of the clinic nurse during this month to troubleshoot any issues.

3. Polysomnography (PSG) Titration (2-months post-op): Also as a component of usual care, two months after surgery, a level-I PSG (in accordance with 2014 American Academy of Sleep Medicine Manual for the Scoring of Sleep and Associated Events) is performed at the Sleep Center with standard parameters. Once asleep, the patient undergoes device titration of the HGNS starting at 0.2V below functional threshold. The voltage is sequentially increased by 0.1V until control of sleep-disordered breathing is achieved, providing "therapeutic amplitude" (treatment).

4B. *Recruitment into the Cardiovascular Outcomes Study (variable months post-op)*: There are two different methods for recruitment:

1. An IRB-approved recruitment letter will be mailed to eligible HGNS patients, which will have contact information for the study team. If interested, the patient will reach out to the PI or the research study coordinator (RSC) who will mail or email (patient preference) a copy of the ICF for review. If contacted by the patient, RSC will obtain verbal consent and will coordinate an in-person visit with the patient at the Penn

Otorhinolaryngology clinic. This visit will include informed consent and visit day one procedures. See 4C below.

2. At the patient's follow-up appointment, Dr. Dedhia will mention the study to the patient and provide a copy of the consent form. Informed consent discussion will be performed at this time. If the patient consents, study day 1 procedures will take place. See 4C below.

- Table 1: Age, gender, race, BMI, smoking status, Pre-op AHI, pre-op ODI, post-op AHI, post-op ODI, Months since implant, Hours of usage since activation, average weekly therapy use since last visit, diabetes status, HTN status, anti-HTN medications.

- Eligibility Source documents: Initial consult note, pre-implant sleep study, pre-implant DISE report, HGNS titration report, HGNS efficacy sleep study report (if applicable), current medications list (reconciled), all Inspire® therapy reports leading up to enrollment.

4C. *Study Timeline – Pages 5 to 10*

- Enrollment (Study Day 1): HGNS therapy will be deactivated (set to 0.0 V) for one week. Sham threshold determination may be done during this visit, or during randomization visit (see below – study week 2). RSC will schedule cardiovascular and sympathetic testing, if possible. The patient will also be instructed about neurobehavioral testing and will perform a practice administration of the DSST and PVT. Form 1 will be completed by RSC (remote #, current therapy status, non-dominant arm diameter, WatchPAT digit determination, medication reconciliation, sham threshold determination (volts), and active therapy setting (volts)). If needed, the patient will contact RSC after the appointment to provide the remote number. The patient will also be given an "on-study card" containing contact information for the research coordinator. The patient will be told to contact the coordinator if any health care providers intend to make changes to the patient's blood pressure medication. An enrollment questionnaire will be completed by the patient:
    - Do you take any medications or substances to improve daytime alertness?
    - Do you take any medications or substances to improve sleep quality?
    - How many hours do you sleep each night, on average?
    - ESS, ISI, FOSQ, Snoring VAS

- Washout Period 1 (Study Week 1): The patient will not use HGNS for 1 week

- Randomization Visit (Study Week 2 with RCD and RSC):
  a. Patient will fill out "washout questionnaire":
    - Patient attestation statement about therapy non-use
    - Have you taken any substance to increase daytime alertness (e.g. caffeine)?
    - Have you taken any substance to improve sleep quality (e.g. alcohol)?
    - Have you had any episodes of falling asleep while driving?

b. Sham Threshold Determination (Consent or Randomization Visit):
Mouth open, nose breathe only, stimulation from 0.1V up by 0.1-0.2V at same electrode configuration as at last clinic visit, level at which bulk tongue motion detected without obvious protrusion. This process is repeated and the average value is used to determine "sham-HGNS". This threshold will be used to define "sub-therapeutic amplitude" (sham). The electrode configuration will remain consistent between the patient's therapeutic and sham thresholds.

c. Randomization: The research coordinator will program the device at the specified amplitude level: either "sham-HGNS" as above, or "therapeutic HGNS," depending on which is assigned first (**see part d below**). The "therapeutic HGNS" setting will be the patient's incoming amplitude on enrollment day which will have been predetermined as part of routine clinical care prior to enrollment in the study. The patient control range will be deactivated for consistency, i.e. there will only be one allowable setting. The research coordinator will instruct the patient to use therapy the same night, and for every night until the next washout after cardiovascular test day (study week 6).

d. Randomization technique: Both patients and investigators will remain blinded regarding patient intervention (sham or treatment HGNS). Only the research coordinator will remain unblinded; he will randomize the patients using a simple scheme (sealedenvelope.com) and will not take part in any assessments. Block design (block size = 10 with 10 units per block) for total of 100 potential subjects. Number 1 = Sham First, Number 2 = Treatment First.

- Blinding Adequacy Phone Call (Week 2, Day 2)
  A blinded member of the study team, as delegated by the PI, will contact the patient and perform a verbal questionnaire:
  1. Do you think you are on "low" or "regular" treatment, or do you not know?
  2. Why you do think so?
     a. Increased snoring per bed partner
     b. Increased fatigue
     c. Morning headache
     d. Nighttime awakening
     e. Other _____

- Weekly Check-In Phone Call (Week 3, Week 4, Week 5)

  RSC weekly call with patient re: usage. Goal is to encourage use (>20h/week)
  RSC will document changes in blood pressure medication, if applicable.
  RSC will remind the patient to consistently take the blood pressure medication. Any questions, comments, concerns, or AEs will be documented at this time as well.

- Overnight testing: 24-hour Ambulatory Blood Pressure Testing (Study Week 6, Day 0-1), and WatchPAT (Study Week 6, Night 1)

1. The patient will be mailed a pre-programmed 24-hour ambulatory blood pressure monitor (ABP) and WatchPAT home sleep test, scheduled to arrive between 2-7 days before physiology testing. For convenience and/or patient preference, the patient may also meet in person with RSC to pick up this equipment. RSC will ensure that ABP, WatchPAT and Inspire® device times are synchronized. Each test will have comprehensive instructions along with a questionnaire (start time, approximate sleep time, wake time, stop time, note anything that may affect the tests). A "blood pressure medication witness" statement will be included in the instructions to ensure that the patient takes the medication on the day of ABP.
2. ABP will be measured using a noninvasive, portable, validated recorder (Spacelab 90227; SpaceLabs Medical; Redmond, WA). The patient will wear the device for 24 hours and asked to perform daily activities as usual. The monitor is programmed to record blood pressure every 30 minutes during the day, and every 1 hour at night. Data are considered valid if at least 50% of the readings over the 24 hours are adequate, with at least 16 hours of use, or at the discretion of the PI. The patient will be asked to record bedtime and wake time to corroborate the sleep and wake time from the recorder. The following night, the patient will perform the WatchPAT test. The patient will be advised not to use both at same time. The patient must use HGNS at same time as WatchPAT. RSC will follow up with patient to ensure both were completed to best of subject's ability.
3. The following day, the patient will return to the lab to return the ABP and WatchPAT equipment and undergo vascular, sympathetic, and neurobehavioral measurements. If any of the ABP or WatchPAT data is invalid or incomplete, the patient may be asked to repeat these tests, at the discretion of the PI. Therefore, the patient will remain at the current setting and will return to have their HGNS reset for therapy washout.

- Vascular Testing (Study Week 6, Day 2): RSC will download therapy compliance data from the patient's remote. Prior to the testing, subjects will have been informed of the pre-visit protocol: no food >6 hours before testing (in the event of afternoon testing, a light, low fat breakfast is permitted); withhold all cardiac and blood pressure medications on the day of testing; no caffeine or alcohol >12 hours before testing; avoid exercise on morning of testing; refrain from cigarette smoke exposure >12 hours before testing; and abstain from vitamin supplementation >72 hours before testing. Upon arrival, the subject will undergo an intake exam including height, weight, vitals, and current medications.
    1. Flow-mediated dilation (FMD) testing. The brachial artery of the non-dominant arm is imaged using a high-resolution 10 MHz ultrasound transducer (Acuson, Siemens AG, Munich, Germany). A blood pressure cuff on the forearm is inflated to suprasystolic pressures to produce 5 min of ischemia. On cuff deflation, imaging is performed to measure FMD. Arterial diameter is measured using a validated software program. Brachial artery FMD is calculated as (post-ischemia diameter − baseline diameter) / (baseline diameter) x 100. The absolute and

percentage increase in brachial diameter in relation to the baseline diameter are examined.

2. Pulse Wave Velocity (PWV) Testing. Following a rest period of 10 minutes with subjects in a supine position in a quiet, temperature-controlled room, the Sphygmocor device® (Atcor Medical, Sydney, Australia) will measure Pulse wave velocity (PWV), augmentation index (AIX), and subendocardial viability ratio (SEVR). The PWV is determined by acquiring waveforms at the carotid and femoral arterial sites with electrocardiogram gating. Velocity (distance/time in m/s) is calculated by measuring the time interval between electrocardiogram R-wave and the recorded waveforms at each site, whereas distance between sites is measured manually. Reproducibility studies at the Emory vascular laboratory on 9 subjects on consecutive days have demonstrated a coefficient of variation of 3.8%, 13.8%, and 20.3% for PWV, SEVR, and AIX, respectively.
3. 24-hour ambulatory blood pressure (ABP). **This test will be done prior to study week 6, day 2 (sympathetic and vascular testing day).** The patient will return the ABP monitor on vascular testing day.

- Sympathetic Measurements (Study Week 6, Day 2): The patient will be laid supine in a comfortable position prior to testing. The 15-minute protocol will involve pre-ejection period (PEP) testing by impedance cardiography.

  Prior to the testing, subjects will have been informed of the pre-visit protocol: no food >6 hours before testing; withhold all cardiac and blood pressure medications on the day of testing; no caffeine or alcohol >12 hours before testing; avoid exercise on morning of testing; refrain from cigarette smoke exposure >12 hours before testing; and abstain from vitamin supplementation >72 hours before testing.

  Pre-ejection Period (PEP): Each patient will be fitted with the Biopac NICO100C Impedance Cardiography (ICG) Noninvasive Cardiac Output Module (Biopac, Goleta CA USA). This device will record the ECG and the ICG continuously during a 10-minute period through eight, disposable, pre-gelled Ag/AgCl electrodes. PEP will be calculated as the average interval (milliseconds) from the onset of left ventricular depolarization, reflected by the Q-wave onset in the ECG to the opening of the aortic valve, reflected by the B-point in the ICG signal.

  - At the end of this visit, the patient will be set to 0.0V for washout period 2.
  - Procedure Day Questionnaire: Therapy attestation statement, medication reconciliation, caffeinated beverages per day, sleep-promoting substance use (e.g. alcohol), ESS, FOSQ, ISI, Snoring VAS.

- Neurobehavioral Measurements (Study Week 6, Day 2): The patient will perform neurobehavioral tests on a designated laptop in a quiet room at the Otorhinolaryngology clinic. Each subject will perform a unique version of the digital symbol substitution test

and psychomotor vigilance test. The laptop is calibrated for timing precision. All patients will be monitored by a trained study staff member to ensure adherence to test procedure protocol.

Prior to testing, subjects will have been informed of pre-visit protocol: abstain from testing if sick; abstain from testing if <1 hour of waking up; and abstain from testing if >16 hours of being awake.

- Washout Period 2 (Study Week 6-7): The patient will not use HGNS for 7 days.

- Crossover Visit & Therapy (Study Week 7): The patient will be switched to the other intervention arm (treatment to sham vs. sham to treatment) for 28 days, or for the duration spent on the previous intervention arm. The research coordinator will program the device at the specified amplitude level, per Form 1.

  Patient will fill out “washout questionnaire #2”:
  - Patient attestation statement about therapy non-use.
  - Have you taken any substance to increase daytime alertness (e.g. caffeine)?
  - Have you taken any substance to improve sleep quality (e.g. alcohol)?
  - Have you had any episodes of falling asleep while driving?

  The research coordinator will reprogram the patient’s HGNS to the amplitude the patient was using prior to study participation. The research coordinator will instruct the patient to use therapy the same night.

- Blinding Adequacy Phone Call (Week 7, Day 2)
  Phone call: second morning questionnaire:
  1. Do you think you are on “low” or “regular” treatment?
  2. Why you do think so?
     a. Increased snoring per bed partner
     b. Increased fatigue
     c. Morning headache
     d. Nighttime awakening
     e. Other _____

- Weekly Check-In Phone Call (Week 8, Week 9, Week 10)

  RSC weekly call with patient re: usage. Goal is to encourage use (>20h/week).
  Document any changes in blood pressure medication.
  Document any AEs, issues or concerns.

- 24-hour Ambulatory Blood Pressure & WatchPAT (Study Week 9, Night 1):

  The patient will be programmed and mailed 24-hour ambulatory blood pressure testing (ABP) and WatchPAT, scheduled to arrive between 2-7 days before physiology testing.

RSC will ensure that ABP, WatchPAT and Inspire® times are synchronized. For convenience and/or patient preference, the patient may meet with RSC in person to pick up the equipment.

- Sympathetic, Vascular, & Neurobehavioral Measurements & Wrap-Up (Study Week 10, Day 2):
    1. RSC will download therapy compliance data from the patient's remote.
    2. The patient will undergo the same set of testing as above.
    3. If any of the ABP or WatchPAT data is invalid or incomplete, the patient may be asked to repeat these tests, at the discretion of the PI. In this case, the patient will remain at the current setting and will need to return to have their HGNS reset to the pre-study amplitude.
    4. Procedure Day Questionnaire: Therapy attestation statement, medication reconciliation, caffeinated beverages per day, sleep-promoting substance use, ESS, FOSQ, ISI, Snoring VAS.
    5. Exit Questionnaire
    6. The patient will resume usual care: the RSC will reprogram the patient's HGNS to the pre-study therapeutic amplitude and will reactivate the stimulation control range. The patient will be encouraged to contact the study team to address any problems or questions.

5. Variables*:*

Medical Chart: age, sex, race, smoking status, hypertension status, diabetes status, apnea-hypopnea index (AHI) from pre-operative sleep study, ODI from pre-operative study, pre-operative DISE report, history of PAP intolerance

Study Data (Clinical): body-mass index, current medications (recorded each study visit), therapy usage (hours/week)

Study Data (Experimental): mean systolic and diastolic blood pressures over 24 hours, mean systolic and diastolic blood pressures during sleep, WatchPAT ODI and AHI from both sham and active therapy tests, pre-ejection period (milliseconds), flow-mediated dilation, peripheral arterial stiffness (PWV in m/s, AIX, SEVR), Snoring Visual Analog Scale (VAS), Epworth Sleepiness Scale (ESS), Insomnia Severity Index (ISI), Functional Outcomes Sleep Questionnaire (FOSQ-10)

6. Analysis

The overall analysis plan is to examine changes in all cardiovascular endpoints over time (i.e., sham to treatment or treatment to sham) as well as internal correlation of these endpoints. Sex, a relevant biological variable, is a covariate in all analyses.

For **Aim 1**, we will perform a paired t-test to compare sham and active therapy 24-hour systolic ambulatory blood pressure values. Improvement in AHI will be calculated as the

difference between pre-operative and post-operative AHI. Multivariable linear regression models will be used to estimate associations between blood pressure reduction (outcome variable) and improvement in AHI (main effect variable) Final models will include age, sex, body-mass index, baseline systolic BP, baseline diastolic BP, antihypertensive medication use.

***H1**: Therapeutic levels of HGNS will reduce mean 24-hour systolic pressures*

***H2:** Therapeutic levels of HGNS will further reduce mean sleep systolic and diastolic pressures*

For Hypotheses 1 & 2, for the purposes of sample size estimation, we are using t-test (bivariate) calculation rather than multivariate analysis. Historical data from an OSA cohort demonstrated an overall 24-hour mean systolic pressure of 133mmHg, with a standard deviation=11. [40] We estimate a clinically significant difference in systolic blood pressure to be 4mmHg[41] which, at the population level, reduces mortality from stroke by 10-40% and mortality from ischemic heart disease or other vascular causes by 7-10%.[42] With significance level $\alpha = 0.05$ (two-tailed) and power $1-\beta = 0.80$, to test a difference in blood pressure of 4mmHg between two repeated measures, a sample of **n=60** would be needed.

For **Aim 3**, we will perform paired t-tests to compare sham and therapy values for both MSNA bursts/minute and pre-ejection period duration. Note that MSNA will no longer be performed at the University of Pennsylvania.

***H1**: Therapeutic levels of HGNS will reduce muscle sympathetic nerve activity, a direct measure of sympathetic activity*

***H2**: Therapeutic levels of HGNS will increase the duration of the pre-ejection period, an indirect measure of sympathetic activity*

For the purposes of sample size estimation for H1, in a published study of OSA patients measuring baseline MSNA, mean MSNA was 39 bursts/min (s.d. = 4). [33] While MSNA is not a commonly used clinical outcome measure, we estimate a significant difference in MSNA to be 5 bursts/min, based on documented effect of CPAP therapy on MSNA. [14] With significance level $\alpha = 0.05$ (two-tailed) and power $1-\beta = 0.80$, to test a difference in MSNA of 5 bursts/min between two repeated measures, a sample of **n=11** would be needed.

For Hypothesis 2, published PEP values in a cohort of OSA patients had a mean of 93 milliseconds (s.d.=17.3). [36] Based on comparative data between untreated apneics and non-apneics, we estimate a significant difference in PEP to be a 10 millisecond increase from baseline. [36] With significance level $\alpha = 0.05$ (two-tailed), power $1-\beta = 0.80$, to test a difference in PEP of 10 milliseconds, a sample of **n=47** would be needed.

For **Aim 4**, we will perform linear regression with MSNA bursts/min and PEP duration as outcome variable and systolic and diastolic blood pressures as main effect variables. We will determine if MSNA and PEP are positively associated with blood pressure measurements while adjusting for confounders of age, sex, body-mass index, baseline systolic BP, baseline diastolic BP, antihypertensive medication use.

***H1:** There will be a significant association between alterations in systolic and diastolic*

blood pressure and muscle sympathetic nerve activity

H2: *There will be a significant association between alterations in systolic and diastolic blood pressure and pre-ejection period duration*

With n=45, we would exceed 95% power to detect effects in the moderate to large range ($r^2 > 0.25$) assuming a two-tailed $\alpha = 0.05$. With smaller effects ($r^2 > 0.16$) power drops somewhat (78%) but still affords a reasonable opportunity to reject the null hypothesis of an association among these various measures of sympathetic function.

For **Aim 5**, we will perform a paired t-test to compare sham and active therapy DSST scores. Improvement in DSST score will be calculated as the difference between the score at therapeutic levels and the score after 30-day sham period. Multivariable linear regression models will be used to estimate associations between DSST score (outcome variable) and improvement in AHI (main effect variable). Final models will include age, sex, body-mass index, baseline DSST.

H1*: DSST score will increase during therapeutic levels of HGNS compared to after the 30-day sham period.*

For **Aim 6**, we will perform a paired t-test to compare sham and active therapy PVT reaction time scores. Improvement in PVT reaction time score will be calculated as the difference between the score at therapeutic levels and the score after 30-day sham period. Multivariable linear regression models will be used to estimate associations between PVT score (outcome variable) and improvement in AHI (main effect variable). Final models will include age, sex, body-mass index, baseline PVT.

H1*: PVT reaction time score will decrease during therapeutic levels of HGNS compared to after the 30-day sham period.*

7. Risk / Benefit

Potential study risks: Patients will undergo laboratory testing of their cardiovascular system following each intervention arm. They will undergo pre-ejection period, by which an external chest monitor and electrocardiogram are used to calculate the time between electrical and mechanical motion of the heart. While muscle sympathetic nerve activity represents an invasive procedure, microneurography is a safe, well-tolerated, standard human research procedure that has been used in multiple studies with a range of patient diagnoses – greater than 200 procedures performed at Emory University by Dr. Jeanie Park, collaborating investigator, without complication. Approximately 7% of subjects have discomfort, including tingling, soreness at the electrode insertion site, or muscle weakness in the leg 1 to 2 days following the procedure; this usually resolves within a week. There are no known long-term risks. Participants are free to discontinue microneurography at any time for any reason.

As part of the experimental study design, the subject will be assigned to a 4-week sham intervention period during which they will most likely experience a recurrence of symptoms of OSA that may have been alleviated by the HGNS during clinical care. As only patients who

had previously untreated OSA therapy are eligible for the study, these patients will experience a return to pre-operative symptoms. We have deliberately excluded patients who were actively using CPAP or other OSA therapy prior to HGNS surgery, as placing these subjects through a period of inadequate treatment (sham period) bears ethical concern. Subjects will be advised to avoid or exercise increased caution when driving or operating heavy machinery during the sham therapy period when they are more likely to feel sleepy. Additionally, patients will wear a blood flow measurement device for 24 hours, which is also well tolerated and does not disturb sleep or other daily activities. Wearing the WatchPAT device while sleeping may result in decreased sleep quality during the night of the testing. However, sleep quality typically returns to usual the following night, with no lasting effects noted.

Potential study benefits: This study is not designed to benefit subjects directly. However, the study results may help doctors to better understand sleep apnea and might be used to help people with sleep apnea in the future.

Risk/Benefit Ratio: As there are few risks in measuring cardiovascular data apart from recurrence of OSA symptoms during the 4-week sham period and there are the possible benefits of better understanding sleep apnea and how to treat people with sleep apnea in the future, the risks of participating in this study are outweighed by the potential benefits.

8. Device Management

Penn owns WatchPAT and ambulatory blood pressure devices, which will be managed, dispensed, and received back from subjects by the study team. Recordings will be handled and scored in accordance with best clinical practices.

9. Feasibility

Sample Size Feasibility: We have recruited 17 subjects (16 have successfully completed the study) since January, 2018, and plan to complete recruitment in 2020.

Technical Feasibility: With regard to Aim 1, 16 subjects successfully completed both sets of 24-hour ambulatory blood pressure testing.

Project execution feasibility: PI has 50% protected research time through 2021. PI will devote 20% of his efforts to ensure the successful completion of this project.

10. Potential Pitfalls, Solutions, and Contingency Plans

1. Study Design

a) The study sample reflects a selective subset of patients with moderate-severe OSA, limiting the generalizability of these findings. Among others, the inclusion criteria are 1) unable to consistently use PAP, and 2) body-mass index < 32 kg/m^2. In clinical practice, this represents only 20% of the moderate-severe OSA population as BMI > 32 kg/m^2 is an exclusion criterion for HGNS and roughly 50% of patients can comply with PAP. From a population health perspective, however, this selected group represents a not insignificant 2% of the U.S. adult population. [4] Note: since this research protocol was created, BMI was

removed as an exclusion criteria for HGNS.

b) We are performing measurements at 2 time points only, immediately after sham and treatment interventions. While we do not capture baseline values, these measures will allow us to compare the individual effects of sham and active therapy. We predict that a 2-week washout period, in contrast to the 1-week withdrawal period in the STAR trial, [23] will provide a return to baseline for ABP at 2 weeks. [31]

c) It is plausible that our hypothesis regarding the positive effect of HGNS on blood pressure is not true for two main reasons. First, we are not restricting inclusion for patients with normal blood pressure. Normotensive patients have been shown to have less improvement in blood pressure, compared to hypertensive patients, after CPAP use. [30] Secondly, our prospective cohort data suggests roughly 50% of subjects will be on anti-hypertensive therapy, potentially masking the blood pressure benefit of HGNS. While we plan to perform multivariate regression controlling for these variables, the sample size will not be sufficient to detect differences in cardiovascular outcomes by subgroup. However, the expected blood pressure values and prevalence of anti-hypertensive medication usage are similar to another OSA cohort in which modest, but significant, improvements were seen with PAP therapy. [43] In exploratory analyses, we can examine patients with baseline hypertension in subgroup analyses.

d) While we have attempted to blind subjects to each study arm, it is possible that experienced HGNS users will recognize the difference between therapeutic and sub-therapeutic HGNS. However, correct guessing of treatment versus sham will not impact the study or the outcomes. Additionally, HGNS users may experience return of untreated OSA symptoms and dropout from the study in order to have therapy restored. Patients will have been thoroughly educated on this aspect of the study prior to enrollment and, as such, we have accounted for 10-15% dropout rate during this period.

e) We plan to include patients with atrial fibrillation while recognizing the potential challenges in measuring pre-ejection period (Aim 2). By using a 10-minute capture period (~600 data points), we anticipate that this will minimize sample variability.

2. Study Results

a) As not all patients will achieve therapeutic success as measured by polysomnography, it is possible that the cardiovascular changes will not be seen in these patients. As part of Aim 1, we will perform linear regression of change in blood pressure upon change in AHI to examine this relationship. As polysomnographic metrics in OSA bear weak a correlation to measures of clinical improvement, [44] it is possible that the relationship between AHI and BP reduction is also discordant. If, in fact, there is a discrepancy between cardiovascular outcomes and polysomnographic outcomes, the findings of this study would challenge the current paradigm in outcome measures for OSA.

b) It is possible that difference between treatment groups is not detected due to the limited sample size (Type II error). This statement applies to Aim 1. If we chose a blood pressure

change of 3mm Hg, instead of 4mm Hg, as a clinically significant difference, our current projected sample size of 60 will be underpowered, 1-β = 0.56. It is noteworthy that the inputs for the sample size and power calculations rely heavily on clinical judgment as poor consensus exists regarding clinically significant differences.

11. Safety and Adverse Events

1. Definitions

a) Adverse Event/Adverse Device Effect: An adverse event (AE)/adverse device effect (ADE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- Is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

b) Serious Adverse Event: Adverse events are classified as serious or non-serious. A serious AE is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- required intervention to prevent permanent impairment or damage
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as non-serious adverse events.

c) Unanticipated Adverse Device Effect (UADE): A UADE is any serious adverse effect on health or safety, or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of

subjects.

2. Recording of Adverse Events

All adverse events occurring during the study period will be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study intervention or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period will be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study intervention or study participation will be recorded and reported.

3. Relationship of AE to Study

The PI will determine the relationship of each adverse event to the study procedures. Relationship will be classified as definitely related, probably related, possibly related, or not related.

4. Reporting of Adverse Events and Unanticipated Problems

The Investigator will promptly notify the Penn IRB of all on-site unanticipated, Adverse Events that are related to the research activity. Other unanticipated problems related to the research involving risk to subjects or others will also be reported promptly. Written reports will be filed using the HS-ERA and in accordance with the Penn IRB timeline of 10 working days.

5. *Follow-up Report*

If an AE has not resolved at the time of the initial report and new information arises that changes the investigator's assessment of the event, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) should be submitted to the IRB. The investigator is responsible for ensuring that all SAEs are followed until either resolved or stable.

12. Management of Multi-Site Research

1. Study Sites:

- University of Pennsylvania
- Thomas Jefferson University

2. Management of Information

The management of multi-site information will be carried out entirely by the research team at the University of Pennsylvania. The research team at Thomas Jefferson University (TJU) will simply provide a list of patients implanted with the Inspire® hypoglossal nerve stimulator and their contact information. All reporting of unanticipated problems, reporting of interim results, and submission of protocol modifications will be executed by the research team at the University of Pennsylvania.

3. Management of Study Teams

The management of study teams will be carried out entirely by the research team at the

University of Pennsylvania. The research team at Thomas Jefferson University will simply provide a list of patients implanted with the Inspire® hypoglossal nerve stimulator and their contact information. All subsequent research-related activities (i.e., consent, enrollment, study procedures, compensation, etc.) will be carried out by the research team at the University of Pennsylvania. For this reason, the research team at the University of Pennsylvania will ensure the research team at Thomas Jefferson University has the most up-to-date version of the study protocol, consent documents, and other supporting materials; however, no study related activities will occur at Thomas Jefferson University.

The only information that will be communicated between the research team at Thomas Jefferson University to the research team at the University of Pennsylvania is a list of patients implanted at Thomas Jefferson University with an Inspire® hypoglossal nerve stimulator and their contact information. This list of PHI will be sent to the research team at the University of Pennsylvania with a HIPAA waiver.

The TJU study team roster and their credentials (e.g., CVs, CITI certificates, medical licenses) will be collected by the Penn research team and kept on file at Penn. TJU will notify Penn of any relevant personnel or credential changes.

Local consent requirements are not expected to vary between study sites, as both sites are located in the same city. However, the research team at TJU will notify the Penn research team of any relevant changes to institutional policy or site-specific circumstances in a timely manner. Penn’s research team will then notify IRB of any resulting changes warranting IRB review.