STUDY PROTOCOL June 23, 2016

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NITROUS OXIDE AS TREATMENT FOR TINNITUS – A PILOT STUDY

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Study Title	Nitrous oxide as treatment for tinnitus – a pilot study
Objective	This pilot study aims to determine if nitrous oxide (laughing gas) is efficacious as treatment for subjective tinnitus.
Study Period	Planned enrollment duration: Approximately 6 months. Planned study duration: 1 year
Number of Patients	40
Study Drug	Nitrous oxide (laughing gas). This is an FDA-approved general anesthetic and sedative agent used in hospitals and dentist offices. The use of nitrous oxide to treat tinnitus is off-label and outside of the approved indication. Nitrous oxide will be administered under supervision of an attending-level anesthesiologist and using standard anesthesia equipment (mobile anesthesia machine, standard peri-procedural monitoring, oxygen, rescue equipment).
Study Design	Prospective, randomized, double-blinded, placebo-controlled cross-over trial. Subjects will first receive either up to 50% nitrous oxide/50% oxygen or "placebo" (50% nitrogen [inert]/50% oxygen) in a 40-minute single session. In the subsequent session, subjects will receive the other treatment intervention. The two sessions will be held 2 weeks apart and will be indistinguishable from each other in setting, setup, and monitoring.
Inclusion and Exclusion Criteria	**Inclusion Criteria** a) Adult men and women 18-65 years of age b) Subjective, unilateral or bilateral, non-pulsatile tinnitus for at least 6 months duration c) Either "Bothered more than a little but not a lot", "Bothered a lot", or "Extremely bothered" on the Global Bothersome scale d) Able to give informed consent e) Must be able to read, write, and understand English **Exclusion Criteria** History of: a) Bipolar disorder b) Schizophrenia c) Schizoaffective disorder d) Substance abuse or dependence (except for remote substance abuse or dependence with remission at least 1 year prior to the study and except for nicotine use disorders) e) Acute medical illness that may pose subject at risk during nitrous oxide administration f) Active psychotic symptoms g) Patients with significant pulmonary disease and/or requiring supplemental oxygen h) Contraindication against the use of nitrous oxide: a. Pneumothorax b. Bowel obstruction c. Middle ear occlusion d. Elevated intracranial pressure e. Chronic cobalamin and/or folate deficiency treated with folic acid or vitamin B12 f. Pregnant patients g. Breastfeeding women i) Previous administration of NMDA-receptor antagonists (e.g., ketamine) within the last 3 months j) Tinnitus related to cochlear implantation, retrocochlear lesion, Meniere's Disease, or other known anatomic lesions of the ear or temporal bone k) Tinnitus related to a Workman's Compensation claim or litigation-related event that is still pending. l) Any medical condition, which, in the opinion of the PI, confounds study results or places the subject at greater risk
Primary Outcome and Measurements	The difference in the difference in patient-reported tinnitus symptoms according to Tinnitus Functional Index (TFI) between baseline and 1 week after each intervention. Secondary outcome measure will be the same calculation performed for the Momentary Assessment of Tinnitus (MAT) questionnaire.

ABSTRACT

Tinnitus, the perception of sound in the absence of external acoustic stimulation, is a common yet poorly understood disease that currently lacks effective front-line treatment. Many therapies focus on mitigating the effects of tinnitus on patients' quality of life, such as through habituation or masking with white noise. However, the tinnitus research literature suggests that NMDA receptor antagonists may prove to be useful in reducing tinnitus itself.[6, 9, 11, 12] Nitrous oxide belongs to the class of NMDA receptor antagonists, and additionally is widely-used, non-invasive, and has a proven safety profile. As such, nitrous oxide may be the drug of choice for testing the efficacy of NMDA receptor antagonists on reducing subjective, bothersome tinnitus.

We propose conducting a pilot randomized placebo-controlled, double-blind crossover study in which patients attend two sessions where they receive either 50% nitrous oxide in oxygen or 50% nitrogen, 50% oxygen for a period of 40 minutes. Tinnitus will be assessed using the Tinnitus Functional Index (TFI) upon enrollment, then reassessed 1 week following each intervention session. Secondarily, the Momentary Assessment of Tinnitus (MAT) questionnaire will be administered at baseline before intervention, 1 hour after intervention, 1 day after intervention, and 1 week after intervention.

1. SPECIFIC AIMS

Aim 1. To evaluate the efficacy of nitrous oxide as treatment for tinnitus

2. BACKGROUND

Subjective, idiopathic, non-pulsatile tinnitus ("tinnitus") is perception of sound without the presence of an external acoustic stimulus. Approximately, 60 million Americans experience chronic tinnitus and 15 million of these people have bothersome tinnitus. Research at Washington University (WU)[1] and elsewhere showed that bothersome tinnitus is associated with poorer working memory, slower processing speeds and reaction times, and deficiencies in selective attention. These findings indicate that tinnitus interferes with attention and other important non-auditory cortical networks and the effects may be mediated by stress and its

effects on the central nervous system.

Currently, no effective front-line therapy exists for tinnitus. Many patients use external sound therapy such as white noise generators to mask the perceived sound, or counseling therapy to habituate the patient to the perceived sound. While these treatments often help decrease the effects tinnitus has on quality of life, they do not alleviate the tinnitus itself. Surgical treatment such as nerve transection remains controversial.[2, 3] Currently, there are no FDA-approved drugs specifically for tinnitus.[4, 5, 6] However, there are antidepressant and antianxiety medications such as desipramine (Norpramin), alprazolam (Xanax), or lorazepam (Ativan) prescribed to patients with tinnitus.[6, 7] According to the American Tinnitus Association, these psychotropic drugs can reduce the psychological burden of tinnitus for some patients, but again do not reduce perception of tinnitus itself. Furthermore, side effects of the drugs include nausea, blurred vision, and insomnia, and they can be habit-forming. As such, the side effects often interfere with patients' ability to concentrate and with other cognitive functions.[7]

A previous trial performed by Dr. Peter Nagele at WU examined using nitrous oxide as a treatment for major depressive disorder (MDD). Results showed significant and rapid improvement of depressive symptoms.[8] Nitrous oxide is an N-methyl-D-aspartate (NMDA) receptor antagonist, a class of drugs that have shown to have antidepressant effects.[9] There is a general consensus in tinnitus research that subjective neural-generated tinnitus results from abnormal spontaneous activity somewhere in the auditory pathway, and the neural events that lead to tinnitus are themselves a result of altered activity in the cochlea.[10, 11] Cochlear NMDA receptors have been implicated in neural-generated tinnitus.[10, 12, 13] Acute excitotoxic tinnitus results from administration of salicylate, but prior administration of NMDA receptor blockers prevents salicylate-induced tinnitus.[12] Given this proposed mechanism of action, nitrous oxide, an NMDA receptor antagonist, presents a possible therapeutic strategy for treating tinnitus.

In this pilot study, we plan to assess the impact of nitrous oxide on tinnitus symptoms and degree of bother. For this pilot study, we will not exclude patients with depression, and thus we will not be able to assess whether the mechanism of nitrous oxide on reducing tinnitus is independent of the aforementioned mechanism in depression. Regardless, we believe nitrous oxide may be an effective treatment for tinnitus and believe a pilot study is indicated.

3. DRUG INFORMATION

Nitrous oxide (laughing gas) is a colorless, odorless gas. Nitrous oxide is the oldest and most widely used FDA-approved anesthetic gas. It is commonly used as a component of general

anesthesia and as a sedative/analgesic agent used in hospitals and dentist offices. The onset as well as offset of effect is within a few minutes. Nitrous oxide is the least potent of inhalational anesthetics. Concentrations above 1atm are theoretically needed to produce complete general anesthesia when nitrous oxide is used as a sole agent. Therefore, concentrations typically used (50%) in dentistry and pediatrics achieve only mild to moderate sedation (but potent analgesia) and are not sufficient to produce general anesthesia. Well-known side effects include euphoria, sedation, nausea and vomiting, and inactivation of vitamin B12 (commensurate with the duration of exposure and concentration used). In general, exposure to 50% nitrous oxide for 40 minutes is considered extremely safe. The use of nitrous oxide to treat tinnitus is off-label and outside of the approved indication.

4. ELIGIBILITY

Inclusion Criteria

a) Adult men and women 18-65 years of age
b) Subjective, unilateral or bilateral, non pulsatile tinnitus for at least 6 months duration
c) Either "Bothered more than a little but not a lot", "Bothered a lot", or "Extremely bothered" on the Global Bothersome scale
d) Able to give informed consent
e) Must be able to read, write, and understand English

Exclusion Criteria

a) History of:
    a. Bipolar disorder
    b. Schizophrenia
    c. Schizoaffective disorder
    d. Substance abuse or dependence (except for remote substance abuse or dependence with remission at least 1 year prior to the study and except for nicotine use disorders)
b) Acute medical illness that may pose subject at risk during nitrous oxide administration
c) Active psychotic symptoms
d) Patients with significant pulmonary disease and/or requiring supplemental oxygen
e) Contraindication against the use of nitrous oxide, including:
    a. Pneumothorax
    b. Bowel obstruction
    c. Middle ear occlusion
    d. Elevated intracranial pressure
    e. Chronic cobalamin and/or folate deficiency treated with folic acid or vitamin B12

f. Pregnancy
g. Breastfeeding women

f) Previous NMDA-receptor antagonist treatment (e.g., ketamine) within the last 3 months
g) Tinnitus related to cochlear implantation, retrocochlear lesion, Meniere's Disease, or other known anatomic lesions of the ear or temporal bone
h) Tinnitus related to a Workman's Compensation claim or litigation-related event that is still pending.
i) Any medical condition, which, in the opinion of the PI, confounds study results or places the subject at greater risk

5. SCREENING AND ENROLLMENT

Participants will be recruited from Washington University School of Medicine Department of Otolaryngology-Head and Neck Surgery clinics, including the Division of Audiology. The Washington University Volunteers for Health Research Participant Registry and Otolaryngology Research Participant Registry will be queried to identify potential participants. Potential participants will be screened according to inclusion and exclusion criteria over the telephone, and those who meet inclusion criteria will be invited to the 8th floor McMillan Building Clinical Research Outcomes Office to discuss study participation. The potential participant will have the opportunity to review the consent form and have questions about the study answered by the study team. The PI, or designee, will obtain written consent prior to the start of study related procedures.

We will enroll a total of 40 participants with bothersome tinnitus to achieve at least 30 evaluable participants. Participants will be offered $50 in remuneration for their time and effort in this study, to be given upon completion of study participation.

6. METHODS

Study Design

This is a prospective, randomized, double-blinded, placebo-controlled crossover trial where 40 patients will undergo two treatment sessions in randomized order. In one session, subjects will receive up to 50% nitrous oxide/50% oxygen for 40 minutes ("Treatment"). In the other session, subjects will receive up to 50% nitrogen [inert]/50% oxygen for 40 minutes ("Placebo"). The two sessions will be held 2 weeks apart and will be indistinguishable from each other in setting, setup, and monitoring.

No pre-enrollment laboratory assessments will be required. However, a health history will be taken at screening.

Randomization

Each participant will be randomly assigned to either the "Treatment first" or "Placebo first" group. The randomization scheme will use permuted blocks of randomly varying sizes generated by the study statistician using SAS statistical software and a password-protected Microsoft Excel spreadsheet will be used to create and store the study IDs and the randomization order. Each block will contain equal numbers of assignments to the treatment first and placebo first in randomized order. To account for the anticipated 20% dropout rate (i.e., as a result of withdrawal, being lost to follow-up, and so on), the randomization table will be generated for 50 subjects.

Blinding

Participants will be blinded to the randomization order. Nitrous oxide is a colorless and odorless gas, which makes it unlikely for participants to identify the group assignment. Likewise, the study setup will be identical for both sessions, which will make an inadvertent unblinding of the study unlikely. For the anesthesiology team responsible for the administration of the nitrous oxide treatment, the group assignment will be known. This team is completely separate from the team assessing the study outcomes, which will be blinded to the treatment received. Treatment administration and outcomes assessment will also be physically separated in different rooms within the Clinical Research Unit (CRU) to prevent unblinding. To assess the integrity of the blind, subjects will be asked immediately after each administration to guess which treatment – active or placebo they just received.

Setting

The study will be performed in the Clinical Research Unit (CRU) at the Center for Applied Research Sciences, located on the 4th and 5th floors of Barnard Hospital. The CRU is staffed by nurses, technicians, and medical assistants with experience in implementing clinical research studies, including specialized protocol-required procedures. The facility consists of 6 rooms and 12 semi-private beds, and is equipped with state-of-the art anesthesia machines, monitoring and anesthesia equipment, and resuscitation equipment and devices. The CRU has anesthesia equipment set up to allow for treatment of two participants at once. We will pay the CRU an annual fee of $500 for this active protocol.

Administration of Study Treatment

Except for the choice of gas (nitrous oxide or nitrogen [inert]; both mixed with 50% oxygen), treatment sessions will be identical. The gas mix will be administered via a standard anesthesia face mask through tubing connected to the inhalation apparatus. Inhaled and exhaled gas concentrations of nitrous oxide, oxygen and nitrogen will be monitored and adjusted according to protocol. Total gas flow will be 2-4 L/min.

Participants will be NPO (although liquids may be freely consumed) for 2 hours prior to each intervention session as a precaution for possible nausea. Participants will be monitored during and after the treatment according to American Society of Anesthesiologists (ASA) standard, which includes continuous 3-lead ECG, pulsoximetry, non-invasive blood pressure and end tidal CO2 under the supervision of an attending-level anesthesiologists.[15] After the 40 minute treatment session, participants will be monitored in the CRU for 1 hour. Participants will then be asked to complete the Momentary Assessment of Tinnitus (MAT) questionnaire before leaving. A study team physician will determine when the participant meets criteria for discharge as described by the ASA standard.

7. DATA COLLECTION

Assessment of Treatment Efficacy

Baseline and Treatment Efficacy Assessment Forms:

1. Demographics
2. Tinnitus Description and History Form
3. Global Bothersome Scale (GBS)
4. Tinnitus Functional Index (TFI)
5. Momentary Assessment of Tinnitus Questionnaire (MAT)
6. Patient Health Questionnaire (PHQ-9)
7. Patients' Global Impression of Change (PGIC)

The effects of the treatment will be assessed using repeat administration of GBS, TFI, MAT, PHQ-9, and PGIC.

Participants will be asked to complete forms 1-6 (detailed above) immediately before each of two sessions as **baseline assessment**, with Demographics and Tinnitus Description and History Form only required before the first session. One hour and then one day following each intervention, participants will complete EMA. One week following each session, participants will complete GBS, TFI, MAT, PHQ-9, and PGIC. These constitute the **treatment efficacy assessment.** In total, each participant will complete the baseline assessment at two timepoints and treatment efficacy assessment at 6 timepoints. The two sessions will be 14 days (2 weeks)

apart. These questionnaires can be completed online via a link provided through REDCap in an email to the participant. At 14 days after administration of nitrous oxide, we expect the effects of nitrous oxide to wear off fully and patients' tinnitus should have returned to baseline. The baseline assessment for the second session will take place no sooner than 14 days after the first session, and therefore will assess whether this washout period of 14 days is accurate. The patient will not be allowed to participate in the second arm of the study until their Global Bother Scale score is within one category of their baseline score.

Assessment of Treatment Safety

Treatment safety will be assessed at several levels: adverse events (AE) related to (i) cardiovascular status; (ii) respiratory function; (iii) central nervous system; (iv) psychiatric symptoms,.

(i) Cardiovascular AEs, such as hyper- and hypotension, tachy-/bradycardia, will be identified by continuous hemodynamic monitoring during and after the treatment
(ii) Respiratory AEs, such as respiratory depression and desaturation, will be identified by continuous pulsoximetry during and after treatment
(iii) Central nervous system AEs will be assessed clinically.
(iv) Psychiatric AEs, will be assessed clinically.

8. DATA AND SAFETY MONITORING

The specific monitoring plan for this study is commensurate with the risks and the size and complexity of the investigations planned. The potential risks are attributable to the use of nitrous oxide. Based on these considerations the monitoring plan involves a DSMC including Dr. Peter Nagele, an attending anesthesiologist knowledgeable in nitrous oxide pharmacology, Dr. Jay Piccirillo, an attending otolaryngologist with experience with tinnitus patients, and Dr. Dorina Kallogjeri, a biostatistician with experience in conducting clinical trials. All reports of Serious Adverse Events or Unexpected Adverse Events will be investigated by the DSMC and reported to the WU HRPO/IRB per reporting guidelines.

Furthermore, the study principal investigator and study coordinator will monitor for breaches of confidentiality and other adverse events on an ongoing basis. Once the PI or study coordinator becomes aware of a reportable adverse event, the event will be reported to HRPO according to institutional guidelines.

9. DATA ANALYSIS

Standard descriptive statistics will describe the study population, responses to assessments, and severity of tinnitus. Data will be analyzed using SAS version 9.4 statistical software (SAS Institute, Inc. Cary, NC).

Aim 1. To evaluate the effect of nitrous oxide as treatment of tinnitus.

The primary efficacy parameter will be the in the change in TFI. Secondary outcome measure will be the change in MAT. **Baseline** TFI and MAT will be defined as those determined immediately before a session. **Post-treatment** TFI and MAT will be compared to **baseline** TFI and MAT at each time point. A mixed model analysis using SAS Proc Mixed procedure will be used to explore the change in TFI score after treatment as compared to after placebo through exploration of the interaction effect of time*treatment. The mixed procedure allows for exploring information from measures nested within subjects nested within treatment groups. In addition, this analytical approach allows for investigation of the carry-over effect due to cross-over design.

And finally, the number of subjects with 13 or more points difference in TFI (clinically important difference)[16] after each treatment arm will be identified and compared between active and placebo treatments using McNemars test.

10. RISK ASSESSMENT

Nitrous oxide. The side effects of nitrous oxide are well known to anesthesiologists. The dose (50%) and duration (40 minutes) used in this protocol are considered extremely safe and of low risk. Similar doses and durations are used in everyday dental practice without vital sign monitoring or supervision by a resuscitation-trained physician. With this dose of nitrous oxide, sedation may occur but not general anesthesia or respiratory depression. Previous evidence shows that the inactivating effect of nitrous oxide on vitamin B12 is dose and duration-dependent and are minor when used as described in this protocol. An increase in plasma homocysteine by +25% and return to baseline within the next 24 hours is expected. Minor side effects after nitrous oxide exposure such as nausea and vomiting may occur which are typically self-limited and short. As a precaution against nausea, patients will be NPO for 2 hours prior to each intervention session, although liquids may be freely consumed. If a patient develops moderate to severe nausea and vomiting, we will administer 4mg of ondansetron.

Questionnaires. Participants may experience frustration and/or boredom completing the questionnaires. The participants may refuse to answer any questions for any reason.

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