RESEARCH PROTOCOL

Closed Loop Acoustic Stimulation during Sedation with Dexmedetomidine (CLASS-D)

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1. SYNOPSIS

Study Title	Closed Loop Acoustic Stimulation during Sedation with Dexmedetomidine (CLASS-D)
Specific Aims	**Specific Aim 1:** Determine whether dexmedetomidine-induced EEG slow waves can be potentiated through in-phase closed-loop acoustic stimulation (CLAS). **Specific Aim 2:** Assess whether performance of a behavioral task during dexmedetomidine sedation is modulated by slow wave CLAS. **Specific Aim 3:** Assess whether thresholds for responsiveness to thermal stimulation during dexmedetomidine sedation are modulated by slow wave CLAS. **Specific Aim 4:** Assess whether dexmedetomidine sedation with CLAS reduces metrics of slow wave need on the night of the intervention. **Specific Aim 5:** Compare modeled brain localization of dexmedetomidine-induced EEG slow waves recorded in the presence and absence of in-phase CLAS.
Study Period	Planned enrollment duration: one year Planned study duration: two years
Sample Size	14 volunteers
Study Design	Prospective within-subject study of dexmedetomidine sedation paired with CLAS conditions in repeated blocks. Intervention will consist of CLAS in-phase with EEG slow waves. Anti-phase stimulation will serve as an active control while sham stimulation will serve as a passive control.
Study Procedures	All participants will receive dexmedetomidine with sedation titrated step-wise to 1, 2, 3 or 4 ng/ml to achieve the induction of EEG slow waves (0.5-4 Hz) while performing a behavioral task (squeezing a ball during inspiration). Subjects will receive multiple 5-minute blocks of acoustic stimulation during two states: 1. while performing the task; 2. after becoming unresponsive to the task. The stimulation blocks consist of: (1) Acoustic stimulation (65 db) synchronized in-phase with the up-slope of EEG slow waves, (2) 65 dB acoustic stimulation synchronized with the down-slope of the EEG slow waves (anti-phase), and (3) sham stimulation (0 dB volume). Quantitative sensory testing (QST) using increasing ramp thermal stimulation (32-52 ºC) will be delivered to compare arousal thresholds between conditions. Unattended home sleep studies will be conducted on the night preceding and following sedation to assess changes in sleep homeostasis. A non-contrast brain MRI will be acquired for localizing EEG slow waves.
Inclusion and Exclusion Criteria	Inclusion: healthy volunteers, 18-40 years old, American Society of Anesthesiologists Physical Status 1-2. Exclusion: Diagnosed sleep disorders, habitually short sleepers, diagnosed psychiatric disorders, use of psychoactive medication (e.g., antidepressants, mood stabilizers or antipsychotics), diagnosed hearing disorder, neck circumference > 40 cm, Body Mass Index > 30, acknowledged recreational drug or nicotine use, resting heart rate during slow wave sleep < 40 beats per minute, pregnancy or nursing, persistently inconsistent or elevated QST heat pain tolerance thresholds (>50 ºC).
Measurements	Primary: EEG slow wave activity (SWA); relative power in the 0.5-4 Hz band. Secondary: Performance of squeezing task, Stanford Sleepiness Scale, Pittsburgh Sleep Quality Index, video recordings, brain MRI, QST heat pain threshold measurements.

Statistical Methodology	SWA across conditions: general mixed-effects models Sleep homeostasis: paired t- or U-tests, depending on normality of measures. Thresholds of responsiveness and task performance: logistic regression modeling. Comparing slow wave topology: Permutation/bootstrap analyses

2. STUDY PROTOCOL

2.1 Background and Significance

Sleep addresses important physiologic needs in humans and most living organisms. Although the neurological mechanisms of sleep are still debated, the electroencephalographic (EEG) correlates of sleep are well characterized.[1] Slow waves, the large amplitude, low frequency EEG oscillations that dominate non-rapid eye movement (NREM) sleep, have been shown to play critical roles in physiologic restoration, memory consolidation and dissipating homeostatic sleep pressure.[2] At the cellular level, these slow oscillations correspond to the synchronous state switching of large ensembles of cortical neurons between depolarized "up" states and hyperpolarized "down" states (i.e. burst firing).[3] At the scalp level, slow wave activity (SWA) is EEG relative power in the 0.5-4 Hz frequency band. SWA is a known marker of sleep need and its dissipation is homeostatically regulated.[4]

Enhancing slow oscillations has recently emerged as a promising strategy to improve natural sleep and treat sleep disorders using electrical, magnetic and acoustic modalities.[5-7] The impact of these interventions on clinical outcomes is an active area of investigation. Preliminary findings have raised the possibility of accelerating the decay of sleep pressure, enhancing memory consolidation and improving autonomic, neuroendocrine and immune function.[8-12] Considering the substantial safety advantages of using sound, acoustic stimulation has recently emerged as the preferred method to enhance sleep slow oscillations in the ambulatory setting.[8,13] These depend on the realtime processing of EEG signal and precise delivery of auditory stimulation in relation to oscillations in the EEG (**Figure 1A**). Over the last decade, multiple algorithms have been developed to synchronize bursts of pink noise in-phase with the up-slope of 0.5-4 Hz slow wave oscillations (**Figure 1B**). Pink noise, also known as 1/f noise (denoting its power density), is random noise that has equal energy per octave. Pink noise acoustic stimuli are hypothesized to transduce through the vestibulocochlear nerve, ascend the non-lemniscal pathway and diffusely recruit neurons across the cortex to depolarize in synchrony with the slow oscillation, reflected by an increase in SWA.[14] Interestingly, antiphase stimulation (i.e. acoustic stimuli synchronized with the down-slope of slow waves) has been shown to disrupt slow wave architecture and decrease SWA.[7] Thus, precisely timed auditory stimulation can either potentiate or undermine the manifestation of EEG slow wave oscillations.

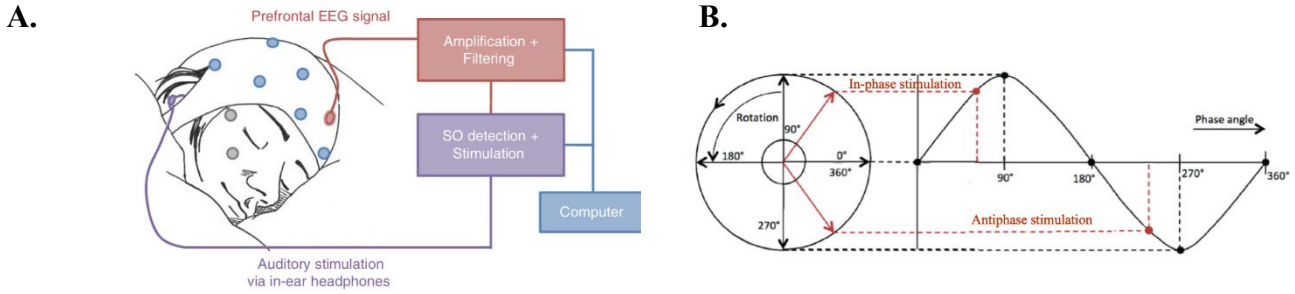

Figure 1. Graphical depiction of closed loop acoustic stimulation. EEG signals are amplified and filtered before undergoing processing. Custom-made scripts detect slow oscillations and compute their oscillatory phase. In-phase acoustic stimuli are delivered during the up-slope of slow waves. Antiphase acoustic stimuli are delivered during the down-slope of slow waves. Adapted from references 9 and 23.

Among sedatives used in clinical practice, dexmedetomidine is posited to induce states most akin to NREM sleep. An alpha-2 agonist, dexmedetomidine induces slow waves and sedation by reducing cerebral noradrenergic tone, similar to the natural sleep onset process.[15] This is in contrast to the majority of anesthetics that also induce slow waves[16] but have a different principal mechanism of action through the GABA receptor. In fact, slow waves generated via dexmedetomidine infusions resemble those of natural sleep.[16,17] The EEG scalp topography of dexmedetomidine sedation has been described using 64-channel EEG[18]; however cortical and subcortical source localization have not yet been undertaken. Moreover, this investigation did not focus specifically on the topology and source estimation of EEG slow waves. The behavioral phenotype of dexmedetomidine sedation is also similar to natural sleep, as patients can be aroused and continue to breathe spontaneously.[19] These properties have made dexmedetomidine a popular sedative in the intensive care setting. Interestingly, SWA has been shown to reflect depth of sedation and probability of arousal.[20,21] Nonetheless, important mechanistic differences between dexmedetomidine sedation and natural sleep exist, and their relationship remains an area of active investigation.[22]

Despite a rapidly growing literature describing the effects of closed-loop acoustic stimulation on slow oscillations during natural sleep, it remains unknown whether these methods can be applied to slow oscillations during sedation. From a scientific perspective, differential effects of acoustic stimulation during sleep and sedation may help shed light on the underlying mechanisms and relationship between these states. The cortical/subcortical sources of EEG slow waves during dexmedetomidine sedation, as well as their effects on sleep homeostasis also remain to be described. From a clinical perspective, non-pharmacologically enhancing slow oscillations during sedation could have tremendous implications. Considering the relationship of SWA with depth of sedation[23], it may be feasible to use lower drug concentrations to achieve similar brain states and behavioral phenotypes. This would be particularly useful when needing deeper states of sedation, or when using agents whose infusion rates are limited by side effects (e.g. dexmedetomidine-induced bradycardia), and in patients who cannot tolerate high doses due to hemodynamic instability or poor physiological reserve. Using lower anesthetic concentrations augmented by auditory stimulation may also impact the incidence and severity of post-operative complications and neurocognitive disorders. Dissipating sleep pressure using sound could be especially impactful on clinical outcomes in the critical care setting, where dexmedetomidine is often used as a chronic sedative.

In summary, closed loop acoustic stimulation can enhance EEG slow oscillations during natural sleep, but whether auditory stimuli can enhance slow oscillations during sedation is unknown. Acoustically enhancing slow oscillations could provide clinicians with a non-pharmacological adjunct to deepen states of sedation, with potential translation toward improved clinical outcomes in the perioperative and critical care settings.

2.1.1 Preliminary Data

There are currently no reported data on the effects of closed-loop acoustic stimulation on pharmacologically-induced slow oscillations. Investigations published in the sleep literature generally report increases in SWA of 10-40%, using phase-locked bursts of pink noise.[7,10-12,24,25]

2.2 Objectives and Specific Aims

The primary objective of this study is to determine whether in-phase CLAS can enhance EEG slow waves during sedation with dexmedetomidine. Secondary objectives are to characterize these potentiated slow waves and their relation to physiologic parameters that define N3 sleep. The corresponding specific aims are as follows:

Specific Aim 1: Determine whether dexmedetomidine-induced EEG slow waves can be potentiated through in-phase closed-loop acoustic stimulation (CLAS).

Specific Aim 2: Assess whether performance of a behavioral task during dexmedetomidine sedation is modulated by slow wave CLAS.

Specific Aim 3: Assess whether thresholds for responsiveness to peripheral thermal stimulation during dexmedetomidine sedation are modulated by slow wave CLAS.

Specific Aim 4: Assess whether dexmedetomidine sedation with CLAS reduces metrics of slow wave need on the night of the intervention.

Specific Aim 5: Compare modeled brain localization of dexmedetomidine-induced EEG slow waves recorded in the presence and absence of in-phase CLAS.

Overall, these data will be expected to address the question of whether in-phase CLAS augments slow wave oscillations during dexmedetomidine sedation. In light of previous studies of acoustic slow wave enhancement, we expect to be adequately powered to detect a 15% difference in SWA between conditions.[8-12] These pilot data will also form the basis for future volunteer and patient studies specifically investigating the prospect of deepening sedation and fulfilling homeostatic sleep needs with CLAS. They will also inform future studies seeking to modulate slow waves during sedation with other agents such as propofol, benzodiazepines, barbiturates, xenon, nitrous oxide, ketamine and halogenated ethers.

2.3 Participant Selection

2.3.1 Inclusion Criteria

Each participant must meet all of the following criteria: 18-40 years old, American Society of Anesthesiologists Physical Status 1-2.

2.3.2 Exclusion Criteria

Participants will not be enrolled if any of the following criteria are fulfilled: diagnosed sleep disorders, habitually short sleepers, diagnosed psychiatric disorders, use of psychoactive medication (e.g., antidepressants, mood stabilizers or antipsychotics), diagnosed hearing disorders, neck circumference > 40 cm, body mass index (BMI) > 30, acknowledged recreational drug or nicotine use, resting heart rate during N3 sleep < 40 beats per minute, pregnant or nursing females. Resting heart rate during N3 sleep will be measured during the pre-sedation sleep study.

2.4. Study Design

For each participant, study participation will focus on a single session of dexmedetomidine sedation flanked by unattended ambulatory sleep studies to assess pre-sedation and post-sedation sleep architecture (**Figure 2**). The post-sedation sleep study will be followed by a brain MRI to allow precise source localization of EEG slow waves recorded during dexmedetomidine sedation and CLAS. The study design will allow investigators to compare the effects of three acoustic stimulation conditions on slow waves in the same brain (within-subject), at the same time of day, and at the same effect-site concentration of dexmedetomidine. The three conditions are: in-phase stimulation (treatment; pink noise bursts synchronized to the up-slope of slow waves), anti-phase stimulation (active control; pink noise bursts synchronized to the down-slope of slow waves), and sham stimulation (passive control; volume set to zero dB, synchronized to the up-slope of slow waves). **Figure 1** graphically depicts the process of closed-loop acoustic stimulation and contrasts in-phase with antiphase stimulation. Washout periods of acoustic silence will be used during the stimulation protocol to control for short-term resonant effects of acoustic stimulation. Based on highly validated and accurate pharmacokinetic models, the rate of dexmedetomidine infusion will be adjusted throughout the experiment to maintain stable plasma and effect site concentrations.

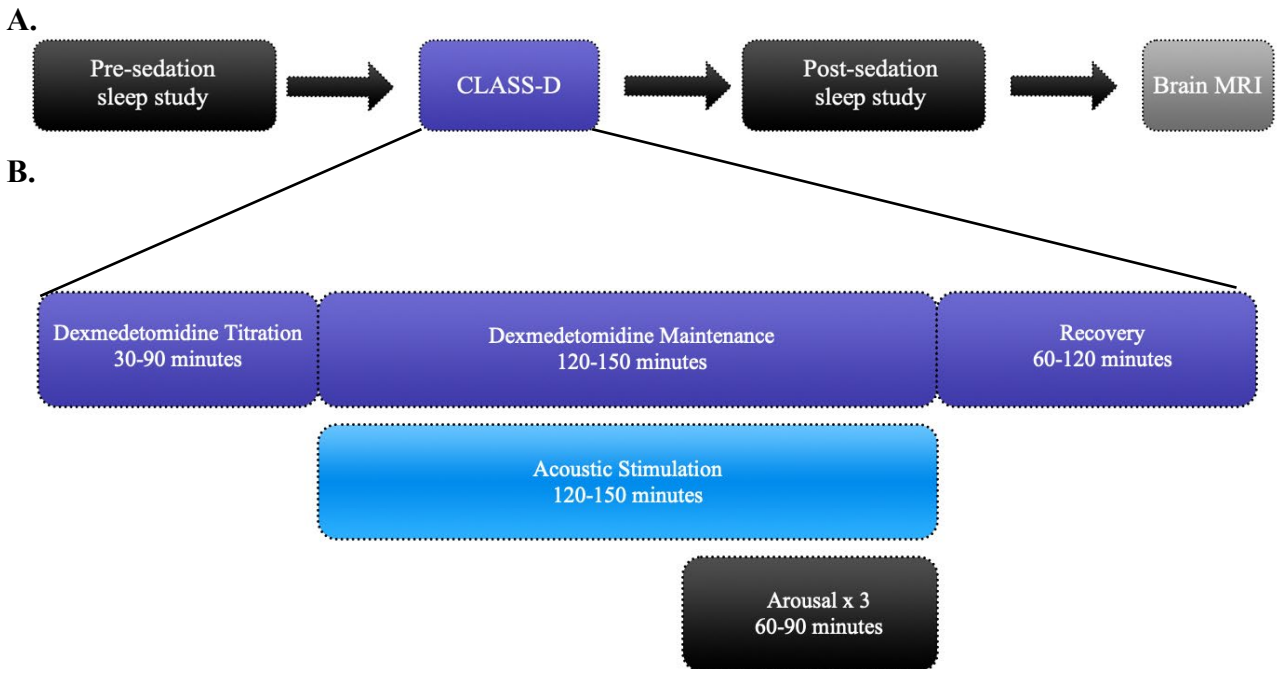

Figure 2. Study design. A. First, participants will complete an ambulatory, unattended home sleep study. The following day, they will undergo the CLASS-D protocol (Figure 4). A second ambulatory sleep study will be completed on the night following CLASS-D. Finally, a subset of participants will complete a brain MRI at a later date. **B.** Graphical depiction of CLASS-D protocol. First, a step-dose infusion of dexmedetomidine is used to induce EEG slow waves and suspend purposeful behavior. Second, 60 one-minute blocks of acoustic stimulation (in-phase, antiphase, sham) are delivered to the participant while the TCI maintains a stable effect site concentration of dexmedetomidine. Third, the participant is aroused once during each stimulation condition (in-phase, antiphase, sham). Fourth, the dexmedetomidine infusion rate is decreased to zero and the participant is monitored as they recover their baseline cognitive and physiological status. The entire CLASS-D protocol is predicted to last between four and six hours for each participant.

2.4.1 Study Procedures

2.4.1.1 – Recruitment, Baseline Testing, and Pre-sedation Sleep

Subjects will be recruited using posted fliers, internet advertisements and Volunteers for Health. Prior to enrolment, subjects will undergo a phone interview to screen for inclusion and exclusion criteria, using an HRPO-approved script.

Subjects who complete the phone screen, are determined to be eligible and are interested in study participation will be scheduled for an initial visit. They will also be asked for their mailing address so a member of the study team can send the informed consent prior to their initial visit. The subject will have time to read the consent, discuss it with their friends and family and ask any questions prior to the start of their first visit when they are asked to sign the informed consent.

Prior to the planned sedation session, participants will be instructed to come to meet research staff at the Department of Anesthesiology (Barnard 2nd Floor Offices, or Peters 2nd or 3rd Floor Offices) or at the Center for Clinical Studies (CCS). They will complete the informed consent at the beginning of this initial visit.

After signing informed consent, each subject will complete a series of questionnaires regarding their sleep and medical history, as well as undergo training by a research coordinator in quantitative sensory testing (QST). Each subject's baseline heat pain threshold (HPT) and heat pain tolerance threshold (HPTT) will be measured with a Thermal Sensory Analyzer (TSA-II, Medoc, Israel) using the method of limits as defined in the German Research Network on Neuropathic Pain's seminal paper describing standardized protocols and reference values.[27] Specifically, the TSA-II device will be applied to the subject's non-dominant volar forearm. The contact area of the thermode is 9 cm^2, and the baseline temperature is 32ºC. Thresholds are obtained by applying ramped thermal stimulation (1 ºC/s) which is terminated when the subject presses a button. HPT is measured by asking the subject to press the button when the initial feeling of warmth transitions to a feeling of pain. HPTT is measured by asking the subject to press the button when they experience intolerable pain. Each threshold (i.e. HPT and HPTT) is measured four times, and the mean of the last three values is considered to represent a threshold. The maximal temperature that can be applied using the TSA-II device is 52 ºC. The maximal thermal energy delivered using this ramping protocol is several orders of magnitude lower that what is required to induce tissue injury. Cumulative equivalent minutes at 43ºC (CEM_{43}) is the standard measurement used to define thermal injury thresholds, and is calculated as: $CEM_{43} = \Delta t R^{(43-T)}$, where Δt is the length of exposure in minutes, T is the average temperature during Δt, and R is 0.25 for $T < 43$ and 0.5 for $T > 43$.[28] The maximal CEM_{43} that can be delivered with the presented protocol is 0.02 (i.e. average temperature of 42ºC over 0.3 minutes), whereas acute minor skin damage begins at a CEM_{43} of 21 (i.e. one thousand-fold higher), and chronic damage at a CEM_{43} of 41. One of the investigators (Dr. Simon Haroutounian) has extensive experience in using the TSA-II to safely determine heat pain tolerance thresholds.[29] Subjects with baseline HPTT > 50ºC will be assessed at a different location on the non-dominant upper extremity. Persistently elevated HPTT > 50ºC will lead to exclusion from the study. Subjects who demonstrate an inability to provide consistent QST responses during pre-screening be excluded from this study. [28]

Each participant will then be provided with a Dreem device, a sleep-monitoring device designed and validated to record ambulatory EEG, heart rate and peripheral oxygen saturation in the home environment.[13] Participants will receive instructions, as well as a link to an instructional video on how to use the device to record ambulatory sleep studies. The principal investigator has extensive experience with this device both in the home setting and in the intensive care unit, in the context of

an ongoing trial investigating post-operative delirium. On multiple nights prior to sedation, each participant will wear the device and physiological data will be recorded (i.e. pre-sedation sleep). On one of the pre-sedation nights, the acoustic stimulation feature of the Dreem will be enabled. This feature is the main feature used by consumers who purchase the Dreem device. To assess the effect of CLAS and dexmedetomidine sedation on subsequent nocturnal sleep architecture, participants will also wear the device on the night following sedation (post-sedation sleep). Participants may also be asked to continuously wear an actigraph watch on their nondominant arm on the days preceding and following the sedation session to objectively measure sleep duration. Participants will also receive standard pre-sedation fasting instructions (NPO 2 hours for clears, 4 hours for liquids, 6 hours for meals) for the dexmedetomdine sedation session.

2.4.1.2 – Simulation and Preparation for Dexmedetomidine Sedation

A target-controlled step-dose infusion of dexmedetomidine based on highly validated pharmacokinetic models will be used to achieve stable plasma concentrations during this study.[20,21,30-32] The target-controlled infusion syringe pump is controlled by Rugloop II©, a Windows® based target controlled infusion and data management program. Rugloop II is not FDA approved for clinical use, however under Code of Federal Regulations Title 21 (21 CFR 812.3(m)) it meets the definition of a non-significant risk device because a) it is not intended as an implant; b) it is not purported nor represented to be for used supporting or sustaining human life; c) it is not for diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health; and d) it does not present a potential for serious risk to the health, safety, or welfare of a subject. While Rugloop-TCI is a computer-controlled device, it delivers the same agent (dexmedetomidine) as a commercial computer controlled infusion pump, delivers it in the same range of infusion rates, and the infusion rate is continuously displayed and continuously monitored by the supervising physician. In addition, the supervising physician has ultimate control over the infusion rate and dose at all times during the dexmedetomidine administration. One of the investigators (Dr. Michael Montana) successfully employed Rugloop II to administer target-controlled infusions of remifentanil in a 70-volunteer study at Washington University Medical Center. There were no reported adverse events during this study.

Prior to each sedation session, Rugloop II will be used to simulate the sedation session for each possible targeted concentration (1, 2, 3 and 4 ng/ml). The individualized drug administration simulation will be reviewed by a board-certified anesthesiologist prior to the sedation session. For use in the critical care setting, the manufacturer's label suggests a dexmedetomidine loading dose regimen of 6 mcg/kg/hr for 10 minutes, followed by lower maintenance infusion rates.[33] Infusion rates are generally limited to 6 mcg/kg/hr in volunteer studies.[18,30,31] These precautions are in place to minimize the risk of symptomatic bradycardia (refer to section "2.5.3 Potential Risks"**).** To minimize the risk of adverse hemodynamic effects, the default maximal infusion rate of dexmedetomidine in Rugloop II is set to 6 mcg/kg/hr. **Figure 3** depicts a simulation for a theoretical participant.

Dexmedetomidine syringes of identical concentration (16 mcg/ml) will be prepared by pharmacy staff at the Barnes-Jewish Hospital Investigational Drug Services, based on an expected maximum total drug administration of 1000 mcg. Alternatively, a board-certified anesthesiologist will prepare syringes as per routine clinical practice and using aseptic procedures.

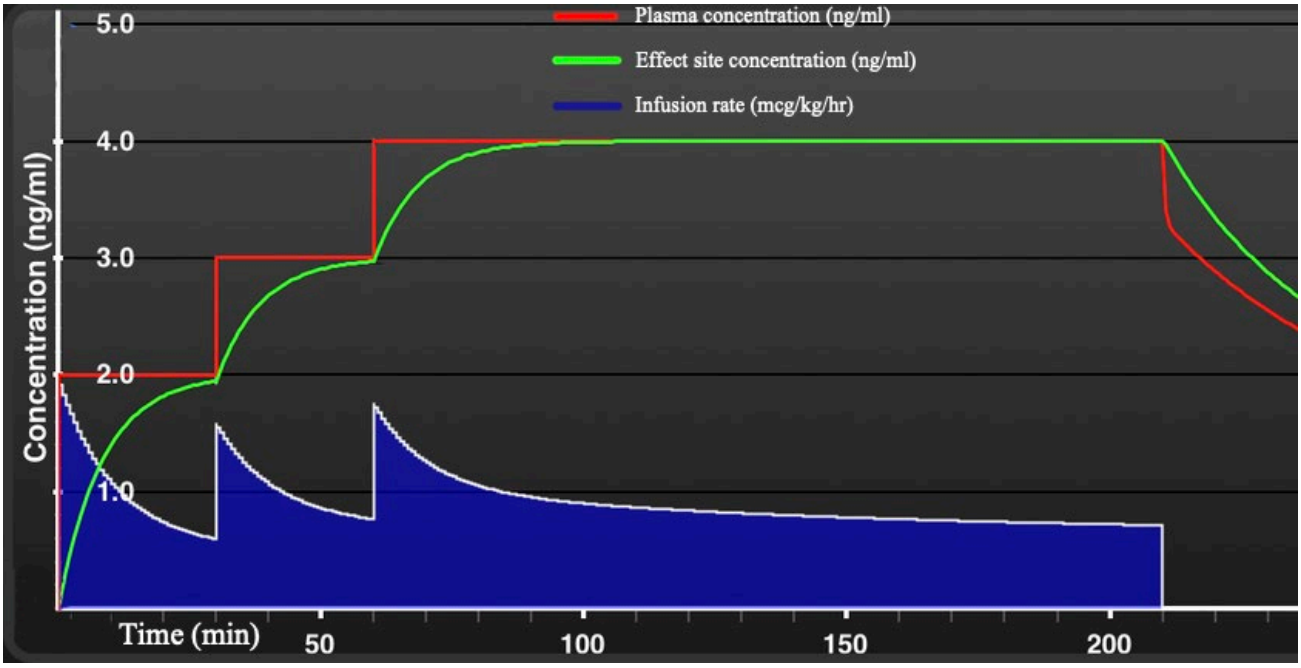

Figure 3. Simulation of a target-controlled step-dose infusion of dexmedetomidine for a 30-year-old man with height of 170 cm and weight of 70 kg. The simulation starts with a target of 2 ng/ml, then increases to a target of 3 ng/ml and finally to a target of 4 ng/ml (maximum target concentration for this study). This simulation represents the highest possible doses of dexmedetomidine for this man during the study. Note that the step-dose titration may also end at 2 ng/ml or 3 ng/ml if both electrophysiological and behavioral criteria are met. The titration phase is followed by an infusion to maintain the targeted concentration during the acoustic stimulation and arousal phases. Finally, the infusion rate is switched to zero during the recovery phase. Recovery of blood samples to measure dexmedetomidine plasma concentrations are depicted by arrows.

2.4.1.3 – Dexmedetomidine Sedation Session

The organization of sedation session phases are depicted in **Figure 2B**.

2.4.1.3.a – Pre-sedation procedures

On the day of the scheduled sedation, the participant will arrive at the Washington University Medical Center and be accompanied to the Department of Anesthesiology. The team will re-administer the screening questionnaire to ensure that all subjects still meet the inclusion/exclusion criteria and that their health status has not changed. Their ambulatory sleep study will be reviewed, and participants with a mean resting heart rate less than 40 bmp during slow wave sleep will be excluded. A complete history and physical examination will be performed. In addition, the staff will ensure that the subject has adhered to ASA fasting guidelines for sedation/general anesthesia (NPO 2 hours for clears, 4 hours for liquids, 6 hours for meals) by the start of dexmedetomidine administration. Pre-sedation sleepiness will be assessed using the Stanford Sleepiness Scale.[34] The participant will then be accompanied to either the post-anesthesia care unit (PACU) or the Clinical Translational Research Unit (CTRU).

Each subject will have two intravenous catheters (IV) placed, one to receive medications and fluid throughout the study protocol, and another to draw blood samples. These may be placed using a small amount of lidocaine to numb the skin prior to the needle insertion if requested by the subject.

Standard ASA monitors will be applied: electrocardiogram, blood pressure cuff, pulse oximeter, breathing rate monitor. Participants will also have a high-density 64-channel EEG scalp electrode net (EGI/Philips) applied with Elefix conductive gel injected within sensors. Additionally, three shielded electrodes will be affixed to the scalp and connected to the custom made CLAS device: one active electrode, one reference electrode, and one ground electrode. The CLAS device consists of EEG amplifiers, a circuit board, a sound card, a visual display, and a battery, all shielded in a metal case and connected to a separate computer for software interfacing. The device receives EEG input, amplifies and filters the signals, tracks the phase of a pre-defined bandwidth (e.g. 0.5-4 Hz) and synchronizes audio output to a the phase specified by the investigators.

Following application of all monitors a QST will be performed to measure HPT and HPTT at the non-dominant forearm (see section 2.4.1.1 for details of QST methods). If HPTT is not greater than 50ºC, the area will be marked. If HPTT is greater than 50ºC, the test will be repeated at a different location on the non-dominant forearm and repeated until the HPTT is found to be less than 50ºC. The final location will then be marked and all subsequent QST measurements will be made at this location. Persistently elevated HPTT > 50ºC will lead to exclusion from the study. EEG data will be recorded during HPPT testing.

The sedation protocol will be completed in a location equipped with standard monitoring, airway equipment, suction, and a board-certified anesthesiologist, per normal standard of practice guidelines. Participants will be instructed to perform a previously validated behavioral task throughout the protocol, while keeping their eyes closed: "squeeze the dynamometer during inspiration and release it during expiration".[35] Participants may also be prompted to complete the task. Participants will be considered responsive whenever they perform the task consistently (at least five consecutive correct squeezes), and unresponsive after five consecutive failures to squeeze during inspiration. After confirming adequate comprehension, 10 minutes of baseline awake EEG measurements will be recorded: five minutes with eyes open and five minutes with eyes closed. After completing all questionnaires and baseline recordings, and ensuring that all monitors are functional, the CLASS-D protocol will begin (**Figure 2B**). The protocol can be broadly separated into four phases: 1. Dexmedetomidine titration phase; 2 Acoustic stimulation phase; 3. Arousal phase; 4. Recovery phase.

2.4.1.3.b – Dexmedetomidine Titration Phase

The participant will be instructed to close their eyes and perform the behavioral task: "squeeze the dynamometer during inspiration and release it during expiration". Participants may also be prompted to perform the task. An initial target concentration of 1 ng/ml will be entered into Rugloop and the infusion will begin. BP, HR and SpO2 data will be streamed to Rugloop. A time stamp will be added to the EEG and video feeds, marking the beginning of the experiment. Once the predicted plasma and effect site concentrations of dexmedetomidine have reached 1 ng/ml, two criteria will be assessed to determine if the experiment may progress to the acoustic stimulation phase. The first criterion is behavioral: investigators will assess if the participant is still performing the behavioral task of squeezing their dominant hand during inspiration. The second criterion is electrophysiological: investigators will visually assess for the presence of large amplitude (> 20 microvolts) slow waves (0.5-4 Hz) on the real-time EEG monitor. The physicians assessing the presence of EEG slow waves have completed accredited training in the interpretation of the EEG of humans undergoing sedation and anesthesia.[36,37] The first state in which participants will received blocks of acoustic stimulation is one

where they remain behaviorally responsive (criteria 1), in the presence of EEG slow waves (criteria 2). If both criteria are met, then the experiment may proceed to the first acoustic stimulation phase. If one or both of the criteria are not met, then the targeted concentration will be up or down-titrated by 0.5 ng/ml on Rugloop II. Once the predicted plasma and effect site concentrations reach the targeted concentration, the two criteria will be reassessed. If both criteria are met, then the experiment may proceed to the acoustic stimulation phase. This process will continue to a minimum target concentration of 1 ng/ml and a maximum of 4 ng/ml. Notably, previous studies have used this step-wise approach to achieve TCI targets as high as 8 ng/ml in order study the neurological and physiological effects of dexmedetomidine.[33] Achieving the desired effect-site concentration may take up to 90 minutes. Level of sedation will be monitored throughout the experiment using the squeeze task described above, video recording, high-density electroencephalography and standard ASA monitors. After completing the first set of acoustic stimulation blocks (see 2.4.1.3.b), dexmedetomidine will be up-titrated to the second state in which participants will receive acoustic stimulation: loss of response on the behavioral task (criteria 1) in the presence of EEG slow waves (criteria 2).

To corroborate and correct the predicted plasma concentrations of dexmedetomidine during the experiment, 5 ml venous blood samples will be collected twice during the experiment: once between the dexmedetomidine titration and second block of acoustic stimulation (once the patient is no longer responding to the behavioral task), and once between the arousal and recovery phases (**Figure 2B**). The sample will be drawn from the peripheral IV not in use for infusing dexmedetomidine. It will be placed on ice prior to processing.

Each blood sample will be cooled and undergo five minutes of spin time in a centrifuge at 3,000 RCF to separate the plasma component. Ethylene diamine triacetic acid (EDTA) will be used as the anticoagulant of choice. Once the plasma has been separated, it will be stored at -70-80 degrees Celsius until being shipped overnight to Denver (CO) on dry ice. Plasma dexmedetomidine levels will be measured from these plasma samples using liquid chromatography-mass spectrometry (LC-MS) at the iC42 Clinical Research and Development Laboratory of the University of Colorado (see letter of support from Dr. Uwe Christians, MD, PhD). Dexmedetomidine will be quantified in 100 µL EDTA plasma, using a validated chromatography-mass spectrometry (LC-MS) assay. One hundred µL EDTA plasma will be transferred into a 96- 1 mL-well plate and a protein precipitation solution (methanol/0.2M $ZnSO_4$, 7:3, v/v) containing the deuterated internal standard will be added. After centrifugation, injection directly from the 96-well plate into the LC/LC-MS/MS system (10 µL injection volume) and online extraction, samples will be back-flushed onto the analytical HPLC column and compounds will be separated using a mobile phase of methanol (containing 0.01% formic acid) and 0.01% formic acid. The HPLC system will be interfaced with a Sciex API4000 tandem quadrupole mass spectrometer *via* a turbo spray source. The mass spectrometer will be run in the positive multiple reaction monitoring (MRM) mode. The assay is validated following current international guidances (FDA, OECD/ICH, CLSI) and has a lower limit of quantification of 100 pg/mL. The range of reliable response is 0.1-500 ng/mL (r^2> 0.99). Inter-assay accuracy is within 85-115% and total imprecision is better than 15% (except at the lower limit of quantitation: <20%). There was no carry-over, ion suppression or matrix interferences. Extracted sample (autosampler) stability for at least 24 hours, long-term storage stability (-80°C) and stability during 3 freeze-thaw cycles has been established.

2.4.1.3.b – Acoustic Stimulation Phase

After confirming that the behavioral and electrophysiological criteria are met, the dexmedetomidine titration acoustic stimulation phases begin. Custom-made scripts in Labview and/or Matlab will track the phase of slow waves (0.5-4Hz) and trigger the binaural delivery of acoustic stimuli via noise-isolating earphones. The acoustic stimuli will be identical to those used in previous sleep studies: bursts of pink 1/f noise of 50 ms duration with a 5 ms rising and falling time.[7,24] Sound volume will be calibrated to 60 dB using a sound level meter. The volume will be reduced if it arouses the participant. The first acoustic stimulation phase, occurring while the patient is still responding to the behavioral task, lasts approximately 30 minutes. The second acoustic stimulation phase, occurring while the patient is not responding to the task, lasts between one and two hours. The acoustic stimulation phases can be conceptually divided into one-minute blocks (**Figure 4**). Prior to the experiment, each block will be assigned to one condition: in-phase stimulation, anti-phase stimulation, sham stimulation, or washout silence. Sequential blocks may be assigned to the same condition (e.g. five consecutive blocks of in-phase stimulation). Washout blocks will precede transitions from one condition to another (e.g. one washout block between blocks of in-phase and anti-phase stimulation). Blocks may be repeated if there are concerns regarding data quality. If there is sufficient scalp space, the Dreem device's acoustic stimulation feature will also be deployed for 15-30 minutes. After completing the acoustic stimulation phases, subjects will enter the arousal phase of the experiment.

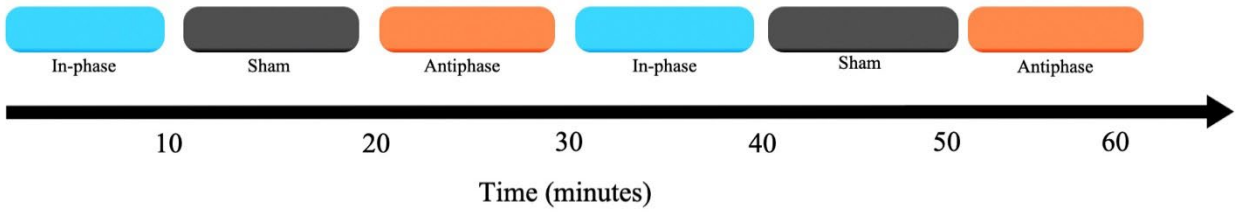

Figure 4. Sample acoustic stimulation protocol. In this sample, a stimulation condition last 9 minutes, transitions into one minute of silence and then into 9 minutes of another stimulation condition. The entire protocol lasts 60 minutes.

2.4.1.3.c – Arousal phase

Subjects will undergo three arousals during this phase of the experiment: one during in-phase stimulation, one during anti-phase stimulation and one during sham stimulation (**Figure 5**). The order of the conditions under which subjects are aroused will be pre-determined before the experiment and balanced across subjects to control for interactions between stimulation conditions and lasting effects of arousal. Each arousal will be preceded by at least 3 consecutive minutes of acoustic stimulation, which will continue during the arousal procedure. The arousal procedure consists of the same ramping thermal stimulation used during pre-sedation QST (see section 2.4.1.1 for methodological details). The cardinal difference between pre-sedation QST and arousal QST is that one of the investigators will be tasked with pressing the button to end the thermal ramp instead of the subject. Criteria for the investigator to press the button will be clear evidence of arousal, specifically, purposeful behavior in response to the thermal stimulation. The investigator will also be able to terminate the thermal stimulation at their clinical discretion (e.g. in the unlikely event that the subject exhibits non-purposeful behavior indicating that they are in pain before any purposeful movement). Vital signs, EEG, and video recording will also be used to review the time of arousal during post-hoc analysis. EEG changes reflective of arousal during dexmedetomidine sedation consist of decreasing SWA and loss of spindles.[20] Assessors of arousal will be blinded to the acoustic stimulation conditions. Once a subject

has been definitively aroused, thermal stimulation will cease and a 15-minute washout period without any thermal or acoustic stimulation will follow to allow the EEG to return to its pre-arousal baseline.[20] Immediately following arousal, subjects will also be asked to report any memories and to rate their pain on a visual analog scale. This protocol will be repeated three times, once for each stimulation condition. The predicted effect-site concentration of dexmedetomidine will be maintained by TCI throughout the acoustic stimulation and arousal phases of the experiment. After the third arousal and washout period, a second 5 ml venous blood sample will be drawn for quantitation of dexmedetomidine concentration and placed on ice for processing. The dexmedetomidine infusion rate will then be set to zero and the recovery phase will begin.

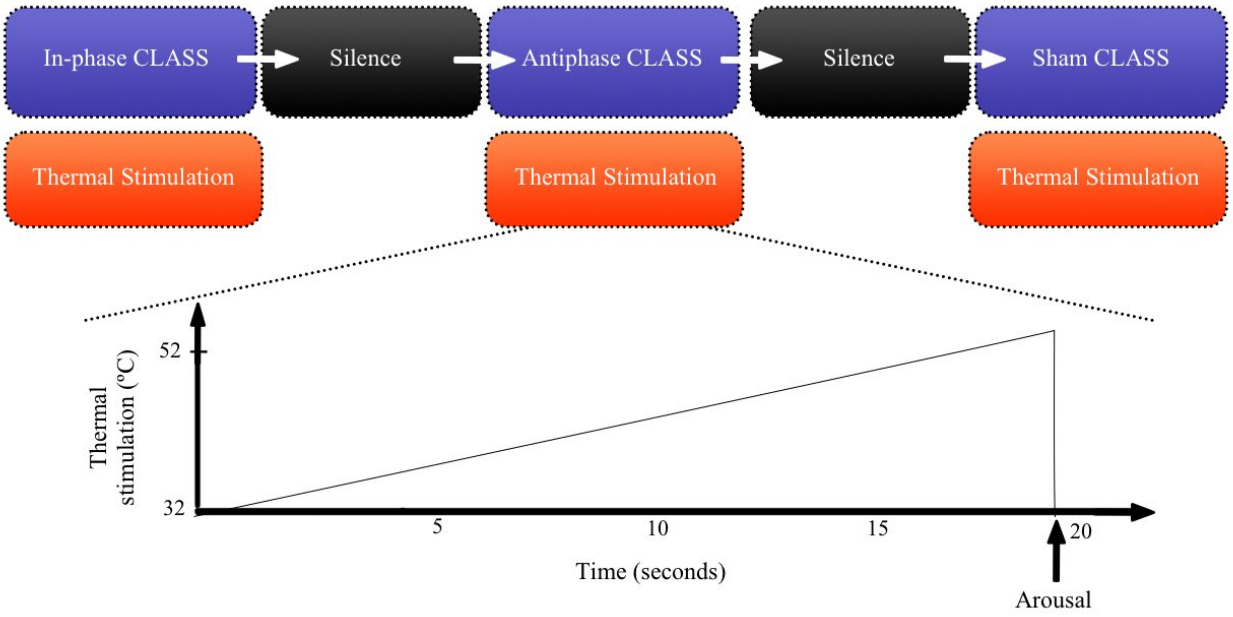

Figure 5. Sample arousal protocol. Ramping thermal stimulation of the non-dominant upper extremity will be used to arouse participants from each of the three CLASS conditions. The temperature (ºC) at which participants are aroused will be compared between conditions to assess whether in-phase CLASS increases arousal thresholds. Fifteen minutes of acoustic and thermal silence will follow each arousal to allow the participant's EEG to return to baseline.

2.4.1.3.d – Recovery Phase

All physiological monitoring will continue during recovery from sedation. Behavioral recovery will be measured with the same paradigm used during induction. Acoustic stimulation conditions (i.e. in-phase vs. antiphase vs. sham) may continue during the recovery phase. When a subject is reliably performing the behavioral task (i.e. squeezing during inspiration, releasing during expiration), they will be considered to be awake and the experiment will terminate. At this time, all acoustic stimulation will cease and the earphones will be removed. The subject may be prompted to perform the behavioral task. The subject will continue to be monitored by staff until they meet standard discharge criteria following sedation. A post-sedation questionnaire will then be administered to assess the participant's memories of the experiment. The Stanford Sleepiness Scale will also be re-administered. The subject will then be

discharged home with the DREEM device to assess structure of sleep on the night of the study. They will also receive standard post-sedation discharge instructions and be accompanied home by a trustworthy person of their choosing.

2.4.1.4 – Post-sedation Sleep and MRI

Participants will be instructed to wear the DREEM and record data during overnight sleep on the night following the sedation protocol. They will return the device the following day and complete a final sleep questionnaire and sleepiness assessment. Participants will also be scheduled for non-contrast brain MRI at a later date. The anatomical data obtained from the individual brain MRI will be used for source localization of EEG slow waves.

2.4.2 Minimization of Bias

The participants and assessors of return of responsiveness will be blinded to the acoustic stimulation conditions.

2.4.3 Pre-Study Period

See section 2.4.1 “Study Procedures”.

2.4.4 Study Period

See section 2.4.1 “Study Procedures”.

Methods:

2.4.6 Observations and Measurements

Measurements acquired for the pre- and post-sedation datasets will include EEG, heart rate (HR), peripheral oxygen saturation (SpO2) and movement. Measurements acquired for the sedation datasets will include EEG, electromyography (EMG), HR, SpO2, respiratory rate, non-invasive blood pressure (NIBP), movement, and audiovisual recordings. Plasma dexmedetomidine levels will also be measured at two time points during the dexmedetomidine infusion. Questionnaires administered include the Stanford Sleepiness Scale and the Pittsburgh Sleep Quality Index.

2.4.6.3 Primary Outcome Measures

Slow wave activity (SWA), cumulative slow wave activity (cSWA).[8]

2.4.6.4 Secondary Outcome Measures

Performance on the behavioral task will be scored as the co-occurrence of hand squeezing and inspiration. The scoring will occur in real-time by one of the investigators. Video footage of participants’ hands and the vital signs monitor will also be used to verify the scoring offline.

Depth of sedation will be measured as the thermal stimulation (ºC) sufficient to arouse subjects, defined as 1) EEG reversion from slow wave oscillations and 2) withdrawal movement or grimacing.

Sleep homeostasis during pre and post-sedation sleep will be assessed via changes in EEG SWA and slow wave features (amplitude, slope) between the first and last sleep cycles of the night, as well as cumulative SWA for the entire night (also called slow wave energy).[8,12,38] De-identified EEG within the Dreem will be uploaded to the manufacturer's website. HDF5 format data will be downloaded and processed using custom-written MATLAB subroutines.

Sleepiness before and after the sedation session will be measured with the Stanford Sleepiness Scale. Subjective sleep quality will be measured using the Pittsburgh Sleep Quality Index.

Structural MRI (T1, MPRAGE scan) and high-density EEG will be used to estimate the neurological sources of EEG slow waves and other motifs of interest during sedation and acoustic stimulation. The models will be generated in Brainstorm[39] running in MATLAB.

2.4.6.5 Statistical Methods

A general mixed effects model will be used to compare primary outcome measures across the three conditions. The model will include time and condition (sham, in-phase, and anti-phase stimulation) as fixed effects and account for age and sex. Paired T tests or U-tests will be used to compare measures of sleep homeostasis between pre- and post-sedation sleep (depending on normality of measures). Logistic regression modelling of responsiveness to thermal stimulation will be used to compare arousal thresholds during in-phase vs. antiphase vs. sham stimulation.

2.4.6.6 Sample Size

A convenience sample size of 14 participants will be used for this pilot study. Previous volunteer studies investigating closed loop acoustic stimulation during sleep have used sample sizes of 11-28 participants to detect changes in SWA of 10-40%.[7-11,13,14,24,25] Based on this literature, we conservatively expect this experiment to be powered to detect a change in SWA of 15%.

2.5 Management of Intercurrent Events

2.5.1 Adverse Experiences

Adverse Event Definition

An adverse event (AE) is any untoward medical occurrence in a subject participating in an investigational study or protocol regardless of causality assessment. An adverse event can be an unfavorable and unintended sign (including an abnormal laboratory finding), symptom, syndrome or disease associated with or occurring during the use of an investigational product whether or not considered related to the investigational product.

These events may be:

a. Definitely related: clearly associated with study drug/treatment
b. Probably related: likely associated with study drug/treatment
c. Possibly related: may be associated with study drug or other treatment
d. Unlikely to be related, or

e. Definitely not related to the study drug/treatment

For reporting purposes, an AE should be regarded as definitely or probably related to the regimen if the investigator believes that at least one of following criteria are met:

a. There is a clinically plausible time sequence between onset of the AE and the administration of the study drug or treatment.
b. There is a biologically plausible mechanism for the study drug or treatment causing or contributing to the AE.
c. The AE cannot be attributed solely to concurrent/underlying illness, other drugs, or procedures.
d. A potential alternative cause does not exist.

Serious Adverse Events (SAE): An adverse drug experience occurring at any dose that results in any of the following outcomes:
a. Death
b. A life-threatening adverse drug experience
c. Inpatient hospitalization or prolongation of existing hospitalization
d. A persistent or significant disability &/or incapacity
e. A congenital anomaly or birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. A serious adverse experience includes any experience that is fatal or immediately life threatening, results in a persistent or significant disability/incapacity, requires or prolongs in-patient hospitalization, cancer, or overdose.

Other important medical events that may not result in death, not be life-threatening, or not require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the subject/patient and may require medical intervention to prevent one of the outcomes listed previously.

Expected adverse events are those adverse events that are listed in the protocol, the Investigator's Brochure (current edition), drug labeling or in the study informed consent document.

These will include relative bradycardia, hypotension, and hypertension.

Unexpected adverse events are those that:
a. are not described in the Investigator's Brochure or drug labeling
b. are not anticipated in the study informed consent. This includes adverse events for which the specificity or severity is not consistent with the description in the informed consent.

Unanticipated problem: Per FDA Procedural Guidance for Clinical Investigators, Sponsors, and IRBs (January 2009), a serious problem is one that has implications for the conduct of the study (requiring a significant and usually safety-related, change in the protocol such as revising inclusion/exclusion criteria or including a new monitoring requirement, informed consent or investigator's brochure).

Unanticipated problem Reporting: Per 21 CFR 312.66, 312.53 (c)(1)(vii), and 56.108(b)(1), should an Unanticipated problem occur during the investigation, the investigator will promptly report all unanticipated problems involving risks to human subjects or others to IRBMED /FDA.

The severity or grade of an adverse event may be measured using the following definitions:
Mild: Noticeable to the subject, but does not interfere with subject's expected daily activities, usually does not require additional therapy or intervention, dose reduction, or discontinuation of the study.
Moderate: Interferes with the subject's expected daily activities, may require some additional therapy or intervention but does not require discontinuation of the study.
Severe: Extremely limits the subject's daily activities and may require discontinuation of study therapy, and/or additional treatment or intervention to resolve.
Event reporting: The study will comply with the IRB & FDA reporting requirements and guidelines.

2.5.2 Premature Discontinuation

Profound treatment-resistant bradycardia, hypertension or hypotension will trigger the premature discontinuation of the sedation protocol. Profound bradycardia will be defined as a heart rate less than 40 beats per minute for more than three minutes. Profound hypotension and hypertension will be defined as a 30% decrease or increase from baseline MAP for more than five minutes, respectively. The inability to maintain a patent airway and/or a sustained decrease in SpO2 below 92% despite the use of simple airway maneuvers and supplementation of up to 4 liters per minute of oxygen will also lead to termination of the sedation protocol. Sustained cardiac conduction abnormalities will also lead to termination of the sedation protocol. The PI reserves the right to discontinue the study protocol at any time for subject safety.

2.5.3 Potential Risks

The most common adverse events associated with dexmedetomidine infusions are bradycardia, hypotension and hypertension. The dose-response relationships of dexmedetomidine with heart rate (HR), mean arterial pressure (MAP) and level of sedation have been rigorously investigated.[31,32] At low plasma concentrations (< 1 ng/ml), dexmedetomidine exerts agonism at alpha2 receptors in the central nervous system, resulting in a decrease in peripheral vascular resistance and heart rate. At higher plasma concentrations (> 4ng/ml), it agonizes alpha2 receptors on vascular smooth muscle, resulting in hypertension and worsening reflex bradycardia. To avoid high peak plasma levels, studies in healthy volunteers generally avoid boluses and limit infusion rates to a maximum of 6 mcg/kg/hr.[17,30] The commonly used loading dose of 1 mcg/kg over 10 minutes (infusion rate of 6 mcg/kg/hr) originates from an early study of dexmedetomidine in healthy volunteers using infusion rates of 1.5, 3, 6 and 12 mcg/kg/hr over 10 minutes.[40] The target effect site concentrations of 2.0-4.0 ng/ml strike a good balance between depth of sedation and hemodynamic effects. Most participants will be moderately sedated, have a near-baseline MAP and a HR > 75% from baseline.[31] It is worth noting that volunteer studies have been safely conducted with target concentrations as high as 8 ng/ml.[30] Furthermore, dexmedetomidine is commonly infused for several hours to days at a rate of 1.5 mcg/kg/hr in intensive care units to critically ill patients, which culminates to a steady-state concentration of 2.5-3.0 ng/ml.

Intravenous fluids, glycopyrrolate, phenylephrine and hydralazine may be used at the discretion of the attending anesthesiologist to treat bradycardia, hypotension and hypertension, respectively. All adverse events will be reported and followed until satisfactory resolution. The description of the adverse experience will include the time of onset, duration, intensity, etiology, relationship to the study drug (none, unlikely, possible, probable, highly probable), and any treatment required. Participant will not be allowed to work or drive until the next day.

2.5.4 Procedures to Minimize Potential Risks

Risks for study subjects will be minimized by initial screening, asking the inclusion/exclusion criteria questions, a complete history and physical by a trained anesthesiologist and urine pregnancy test, ASA standard monitoring and standard fasting guidelines.[41] A staff anesthesiologist or anesthesiology resident will be present at all times. An ACLS-certified anesthesiologist will be immediately available.

Steps will be taken to minimize the risk of drug administration errors. Pharmacy staff at the Barnes-Jewish Hospital Investigational Drug Services will prepare dexmedetomidine drug syringes for each session. Alternatively, a board-certified anesthesiologist will prepare syringes of these concentrations and volumes, as per routine clinical practice and using aseptic procedures. The study team has designated a standard of syringes filled with dexmedetomidine at 16 mcg/ml for all study sessions. The working concentration is four times the standard working concentration used in the OR and ICU settings and is based on a projected maximal dexmedetomdine administration of 1000 mcg for an entire session (maximal target infusion rate for plasma concentration of 4 ng/ml, 90 kg male). The use of standardized drug syringe type, volume, and working concentration is designed to maximize participant safety.

Decades of use have shown that target-controlled infusion devices are a mature and safe technology with over 90 published articles using Rugloop-TCI.[42] A series of recently published studies have safely used Rugloop-TCI to achieve dexmedetomidine plasma concentrations as high as 8 ng/ml, twice as high as the maximal concentration proposed in our study.[30-32] The use of a target controlled infusion device delivers the same medication (dexmedetomidine) over the same range of infusions as a continuous infusion pump. The supervising physician ultimately determines the infusion rate and the device continuously displays the infusion rate. In addition, the sedation session will be conducted with continuous vital sign monitoring by an anesthesia provider. In the unlikely event that they are needed, drugs and equipment to treat any anticipated or unanticipated side effects will be immediately available. Resuscitation capabilities will be immediately available. Following completion of the dexmedetomidine infusion, participants will be monitored until they return to their baseline mental status as assessed by a specialty trained anesthesiologist, and will be instructed not to drive themselves home.

The maximal thermal energy that can be delivered during pre-sedation QST and the arousal phases of this experiment is a thousand-fold lower than what is required to induce acute mild tissue injury (see section 4.2.1.1 for details on methodology and injury thresholds).

To minimize breach of confidentiality risk, the minimum necessary data will be collected to achieve the study objectives, datasets will be de-identified after study completion, and data and code keys will be stored in password-protected databases and key-locked filing cabinets.

2.5.5 Data and Safety Monitoring Plan

The specific monitoring plan for this investigation is commensurate with the risks and the size and complexity of the studies planned. The PI will monitor the study for any adverse and serious events. Based on the small size and relatively low risks nature of the protocol, an anesthesiologist without current collaborations with the study team will also review the study in lieu of a Data Safety Monitoring Board. All serious events will be reported to the HRPO within 7 days. Should a serious adverse event occur the study will be stopped. An investigation will be conducted and finding report generated before the study is resumed. After seven participants have been enrolled, the study will undergo a planned audit by the Anesthesiology's Division of Clinical and Translational Research for quality assurance and safety assessment and the anesthesiologist monitoring the study.

3. HUMAN SUBJECTS RESEARCH

3.1 Protection of Human Subjects

The study will be conducted with strict adherence to Washington University Institutional Review Board protocol and consent form approval. An American Board of Anesthesiology board-certified and GCP-certified anesthesiologist with experience in conducting clinical studies will lead the study. Safety and privacy of study participants will be safeguarded.

Participant confidentiality will be maintained through de-identification of personal health information. Identity and linking information will be locked cabinet within the principal investigator's (PI) office, which is locked outside of business hours. Electronic data will be password encrypted on secure servers.

3.2 Sources of Materials

Collected materials will be restricted to a brain MRI, video recordings collected during experimental sessions and physiological data collected during physiological sessions as well as those collected by the Dreem device during sleep at home. These physiological data include EEG, EMG, SpO2, HR, NIBP, and movement.

Participants will receive remuneration of $180 per sedation session and $10 per home sleep study with Dreem (maximum of two), and $50 for an MRI scan, or $250 for completion of the study. There will be no cost for involvement.

3.2.1 List of Protected Health Information Collected for Study

The following HIPAA protected identifiers will be collected during the study: name, birth date, geographic address, email address, telephone number. This information will be collected to ensure adequate communication between experimental sessions and to retrieve the Dreem devices. Video

recordings will also be collected during the experimental sessions. All data will be deidentified when the study is complete.

3.2.2 Data Management

Physical data will be stored in a locked cabinet within the PI's office, which is locked outside of business hours. Electronic data will be password encrypted on secure servers.

3.3 Recruitment and Informed Consent

Subjects will be recruited using posted fliers and Volunteers for Health. Recruitment flyers will be placed across the Washington University Danforth and Medical campuses. Screening and enrollment of subjects will occur according to the protocol-defined inclusion and exclusion criteria. The PI, collaborators or research assistant will obtain informed consent.

3.4 Potential Benefits of the Proposed Research to the Subjects and Others

There is no benefit to individual subjects in this study. Society may benefit from a better understanding of the interaction between dexmedetomidine sedation and sleep homeostasis. Discovering a method to non-pharmacologically enhance sedation stands to benefit countless patients who require sedation for invasive procedures and during critical illness.

3.5 Inclusion of Women

Efforts will be made to enroll participants regardless of gender.

3.6 Inclusion of Minorities

All studies in the Department of Anesthesiology encourage the participation of minorities in research.

3.7 Inclusion of Children

Individuals of less than 18 years of age will not be enrolled.

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