Repurposing Dexmedetomidine as an Orally Administered Sleep Therapeutic

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I. Background and Significance

Challenges in Drug Discovery

Basic science discoveries of the molecular basis of disease have the potential to provide immeasurable opportunities to translate discoveries into targeted therapeutics (Collins 2011). However, translation of these basic science discoveries to targeted therapeutics is often met with failure (Collins 2011). A paradigm shifting approach (complementing current approaches) to reposition drug discovery efforts from an era of 'me-too' or 'slightly me-better' drugs to one of highly innovative medicines is necessary (Paul, Mytelka et al. 2010). Presently, we know the molecular basis of approximately 4,000 disorders, however targeted therapies for only 250 exist (Collins 2012). This mismatch is present because the traditional approach to drug discovery involves de novo identification and validation of new molecular entities, which is a time-consuming and costly process (Paul, Mytelka et al. 2010). Despite huge investments in drug discovery and development, the number of new drugs introduced into clinical practice has not increased significantly. For example, while the total research and development expenditure for drug discovery worldwide increased 10 times from 1975 ($4 billion) to 2009 ($40 billion), the number of new molecular entities approved has remained stagnant (Paul, Mytelka et al. 2010). Drug discovery failure rates can be as high as 95% and the average time from target selection to approval is approximately 13-14 years (Paul, Mytelka et al. 2010). When failures are accounted for, the cost of bringing a new drug to the market is approximately $2 billion (Paul, Mytelka et al. 2010). Therefore, strategies to reduce the average time for drug approval, decrease costs, and improve success rates are urgently needed. Drug rescue and repurposing can be one of those strategies, as it offers the key advantage of harnessing previous research and development efforts (Paul, Mytelka et al. 2010, Collins 2011). Approved drugs and many abandoned compounds have already been tested in humans, and detailed information is available on their pharmacodynamics, pharmacokinetics, formulation, dosing, and potential toxicity (Paul, Mytelka et al. 2010, Collins 2011). Since drug repurposing is built upon previous research and development efforts, new candidate therapies are ready for clinical trials in a very timely fashion, speeding their review by the Food and Drug Administration and their integration into clinical practice (Paul, Mytelka et al. 2010, Collins 2011).

Neurophysiological Principled Approach to Drug Discovery and Repurposing

Neural oscillations form well-orchestrated structure for communication or miscommunication within and across neural circuits at multiple time scales (Buzsaki and Freeman 2015). It has been postulated that in the next decade, studies of neural oscillations are expected to spearhead insights on brain function and provide a much needed alternative approach to the development of novel therapies (Buzsaki and Freeman 2015). Today, studies of neural oscillations are a significant component of systems neuroscience research. In humans, valuable insights into neural oscillations and neural circuit mechanisms of altered arousal come from our clinical and research experiences in anesthesiology. In our systems neuroscience studies of anesthesia, we have found that neural oscillations observed during altered states of arousal possess structured phenotypes that are appropriate for obtaining mechanistic insights into mechanisms such as sleep (Ching, Cimenser et al. 2010, Purdon, Pierce et al. 2013, Vijayan, Ching et al. 2013, Akeju, Pavone et al. 2014). In my research laboratory, we are studying the functional characteristics and neuroanatomical basis of these oscillations, and relating them to normal and pathological brain functioning. We expect that the techniques and insights emanating from this neural oscillation focused approach will serve important areas in basic and clinical neuroscience especially sleep medicine.

Mechanisms of Natural Sleep

Sleep is a basic human function that occupies approximately one-third of our lives, yet science is not entirely clear on its purpose (Brown, Basheer et al. 2012). Although the purpose of sleep is not entirely clear, great progress has been made in understanding the neural oscillations and circuitry that control sleep (Brown, Basheer et al. 2012). During natural sleep, humans switch at approximately 90-minute intervals between two states: rapid eye movement sleep (REM) and non-REM sleep. This oscillatory dynamic is postulated to be fundamental for achieving the restorative benefits of natural sleep. Non-REM sleep is initiated when the preoptic area (POA) activates and thereby, blocks the arousal inputs of these centers to the thalamus and the cortex (Saper, Fuller et al. 2010). With the initiation of this inhibition, the EEG shows intermittent sigma/spindle activity (9–16 Hz oscillations) followed shortly thereafter, by slow-wave (< 1 Hz) and delta (1-4 Hz) oscillations

(Saper, Fuller et al. 2010). Non-REM II sleep is defined by the presence of spindles with slow waves and K-complexes (Saper, Fuller et al. 2010). Spindling is believed to be intermittent rhythmic activity between the cortex and the thalamus that results from this inhibitory state (Saper, Fuller et al. 2010). Non-REM stage III sleep is characterized by a predominance of slow-delta EEG oscillations (Saper, Fuller et al. 2010). The slow-delta oscillations likely result from cortical hyperpolarization due to decreased excitation from the arousal centers and the thalamus. The transition from non-REM sleep to REM sleep is initiated when the cholinergic centers in the pedunculopontine tegmentum (PPT) and the laterodorsal tegmentum (LDT) activate. Activity of the monoaminergic dorsal raphé and locus coeruleus (LC) during non-REM sleep inhibits the PPT and LDT suppressing REM sleep (Saper, Fuller et al. 2010). The dorsal raphé and LC cease firing at REM onset. Activation of the PPT and the LDT, which project to the thalamus, basal forebrain and to the cortex, results in the switch of the EEG from slow-wave-delta oscillations to the awake-like EEG patterns characteristic of REM sleep. Non-REM sleep represents periods of cortical quiescence that likely allow the brain to restore its energy levels and has been strongly linked to improved memory function (Stickgold 2005). REM sleep may play a critical role in memory consolidation (Stickgold 2005).

Insomnia: A Clinical Problem With Healthcare Implications

Much of what is known about the benefits of sleep in humans has been obtained from studies of patients with insomnia. Insomnia is defined as a repeated difficulty with sleep initiation, duration, consolidation, or quality that occurs despite adequate time and opportunity for sleep and results in some form of daytime impairment (American Psychiatric Association. 2013). Insomnia is the most common sleep disorder, with a reported prevalence of 10 to 15%, depending on the diagnostic criteria used (Winkelman 2015). Roughly 50% of patients with insomnia have a psychiatric disorder, most commonly a mood disorder (e.g., major depressive disorder) or an anxiety disorder (e.g., generalized anxiety disorder or post-traumatic stress disorder) (Winkelman 2015). Although roughly 80% of those with major depressive disorder have insomnia (insomnia doubles the risk factor for developing depression), in approximately half of those cases, insomnia predated the onset of the mood disorder (Winkelman 2015). This suggests that insomnia is an independent risk factor for major depressive disorder. Insomnia is also an independent risk factor for acute myocardial infarction and coronary heart disease, heart failure, hypertension, diabetes, and death (Winkelman 2015). Unfortunately, at present, the medicines currently used to treat insomnia are associated with side effects such as daytime sedation, delirium, ataxia, anterograde memory disturbance, and complex sleep-related behaviors (Wafford and Ebert 2008, Winkelman 2015). More so, sleep disturbances may also lead to delirium and other cognitive deficits such as psychosis (Maldonado 2013). Thus, an effective treatment for insomnia will have immense public health benefits.

Current Sleep Therapeutics Do Not Approximate Natural Sleep

In the United States, insomnia therapies, ranging from prescription to over-the-counter medications, are a multi-billion dollar market with numerous pharmacological targets for the treatment of insomnia. The principal sleep medications (i.e. benzodiazepines, eszopiclone, and zolpidem) are among the most widely sold pharmaceuticals (Wafford and Ebert 2008). These drugs target inhibitory gamma amino-butyric acid A ($GABA_A$) receptors throughout the brain. The drug formulations are designed for immediate release or timed release based on whether the insomnia is due to difficulty falling asleep or difficulty staying asleep respectively. They cause changes in the normal sleep architecture, including increased non-REM II sleep and increased REM latency. Importantly, these medications do not approximate natural sleep or selectively activate the brain circuits in a precisely timed manner to drive the 90-minute non-REM-REM cycling of natural sleep required for its restorative benefits. Instead, these medications globally inhibit brain activity, and at best, produce only sedation. Sedation with these sleep medications is associated with beta (13-25 Hz) oscillations (van Lier, Drinkenburg et al. 2004), an oscillatory dynamic that is distinct from non-REM and REM sleep, and is responsible for the amnesic effects of GABAergic drugs (Feshchenko, Veselis et al. 1997). Thus it is not surprising that principal sleep medications possess adverse side effects such as memory and cognitive impairment (Wafford and Ebert 2008, Winkelman 2015).

Other agents: Ramelteon selectively targets hypothalamic melatonin receptors involved in the sleep–wake cycle. Studies suggest it significantly shortens sleep onset and increases sleep time without evidence of rebound insomnia, but it is associated with somnolence, fatigue, and dizziness (Wafford and Ebert 2008). Melatonin is also used as an insomnia therapy but there is minimal data supporting its efficacy (Wafford and Ebert 2008). Other medications such as antidepressants are often used off-label when treating insomnia. Nonprescription sleeping aids include antihistamines, though much of the existing data does not support their

benefit for insomnia, and subjects quickly develop tolerance to sedative effects or rebound insomnia upon discontinuation (Wafford and Ebert 2008). The hypocretin/orexin system involved in narcolepsy was recently identified as a possible target (Wafford and Ebert 2008), and led to the development of the newest sleep therapeutic suvorexant. However, this drug has also been associated with adverse side effects such as memory and cognitive impairment, and suicidal thoughts (Jacobson, Callander et al. 2014). Thus, there is a great clinical need to develop more efficacious agents that closely approximates natural sleep with better side effect profiles. Given the high prevalence of insomnia and known diseases that are precipitated and/or aggravated by sleep disturbances such as depression, cardiovascular disease, diabetes, and cognitive impairments, a novel and effective medication for sleep will have immediate and immense public health benefit.

Evidence For Repurposing Dexmedetomidine as a Sleep Therapeutic

Dexmedetomidine is an alpha-2 adrenergic agonist that is typically administered as an anesthetic adjunct. Patients who are sedated with dexmedetomidine are arousable and responsive in a manner that is very similar to that seen in people who are sleeping (Cortinez, Hsu et al. 2004, Akeju, Pavone et al. 2014). Dexmedetomidine is thought to alter arousal primarily through its actions on pre-synaptic alpha-2A adrenergic receptors on neurons projecting from the LC (Correa-Sales, Rabin et al. 1992, Chiu, Chen et al. 1995). Dexmedetomidine binding hyperpolarizes LC neurons, decreasing norepinephrine release (Jorm and Stamford 1993, Nacif-Coelho, Correa-Sales et al. 1994, Nelson, Lu et al. 2003). Hyperpolarization of LC neurons results in loss of inhibitory inputs to the pre-optic area of the hypothalamus and excitatory inputs to the cortex, basal forebrain and the central thalamus. The pre-optic area sends GABAergic and galanergic inhibitory projections to the major arousals centers in the midbrain, pons and hypothalamus (Saper, Fuller et al. 2010). Hence, loss of inputs from the LC is thought to result in sleep-like state (Carter, Yizhar et al. 2010), due to activation of inhibitory pathways from the pre-optic area to the arousal centers along with loss of excitatory inputs to the cortex, basal forebrain and the central thalamus. Not surprisingly, as in non-REM II sleep, spindles and slow oscillations are associated with the altered state of arousal that is induced by dexmedetomidine (Akeju, Pavone et al. 2014). It is important to note the behavioral and neurophysiologic similarities between dexmedetomidine-induced altered arousal and sleep occurs because activation of inhibitory inputs from the pre-optic area is an essential component of how non-REM sleep is initiated (Sherin, Elmquist et al. 1998, Morairty, Rainnie et al. 2004, Saper, Fuller et al. 2010).

We recently conducted a clinical trial (NCT01485380) that utilized a combination of high-density EEG and Fludeoxyglucose Positron Emission Tomography/Magnetic Resonance (PET/MR) imaging to study the brain states induced by dexmedetomidine. Results from this clinical trial of dexmedetomidine suggests that this drug may be used to induce and maintain a brain state that closely approximates non-REM II sleep state characterized by sleep spindles, and slow-delta oscillations (Akeju, Pavone et al. 2014). Similar to non-REM sleep, we also found that dexmedetomidine decreased cerebral blood flow and cerebral metabolism, but maintained cortico-cortical functional connectivity patterns (Akeju, Loggia et al. 2014). These results lend to a very specific rhythms based hypothesis of the mechanisms necessary to engage sleep. Can successful sleep state switching be accomplished in humans by inducing a non-REM II state and then allowing indigenous sleep mechanism to engage normal sleep cycling. Until now, it has been unclear whether any pharmacological agent can induce a state that closely approximates natural sleep or sleep state switching. The possibility that dexmedetomidine could be used as a sleep therapy has not been readily apparent because it is currently marketed as an intravenous drug. Furthermore, a systems neuroscience approach to studying the dexmedetomidine-induced brain state had previously not been accomplished. A key strength of this proposal is that it is based upon compelling results obtained from our recently conducted Phase I/II proof-of-concept clinical study (NCT01485393) where we established the safety, efficacy, dosing paradigm, and dose for successfully using dexmedetomidine to induce sleep, which is characterized by non-REM I-III and REM sleep states. Therefore, we hypothesize that oral dexmedetomidine will shorten sleep latency by inducing a non-REM II state that will allow indigenous sleep mechanisms to engage successful sleep state switching into non-REM III and REM sleep states, conferring the restorative benefits of sleep.

Currently dexmedetomidine is not available in an oral or sub-lingual preparation. However, the intravenous form of dexmedetomidine has a buccal bioavailability of approximately 86% and an oral bioavailability of approximately 16% (Anttila, Penttila et al. 2003). In our clinical practice of anesthesiology, the oral solution of dexmedetomidine, which is the intravenous formulation administered at up to 5 times the typical intravenous

bolus dose of 1mcg/kg has been used off-label and shown to be effective as a sedative (Ray and Tobias 2008, Mountain, Smithson et al. 2011). Thus, we expect that the oral reformulations of dexmedetomidine will be bioavailable and efficacious. Importantly, the oral administration of dexmedetomidine solution at up to 5 times of the recommended bolus dose does not appear to result in either of the two commonly reported side effects of hypotension and bradycardia that are associated with intravenous administration of dexmedetomidine (Ray and Tobias 2008, Mountain, Smithson et al. 2011). Therefore, we expect oral dexmedetomidine to be efficacious and safe for administration in humans.

In summary, existing sleep medications are non-specific sedatives with significant cognitive side effects. However, our systems neuroscience based studies and proof-of-concept studies strongly suggest that dexmedetomidine is biochemically and neurophysiologically well suited to engage the natural mechanisms that drive sleep. Thus, this study will demonstrate a putative new class of sleep therapeutics to treat insomnia. If this research is successful, we will see a resolution to the problem of morbidity caused by insomnia and side effects of current sleep therapeutics.

II. Specific Aims:

The broad objective of this investigation is to assess the safety and efficacy of oral therapy with dexmedetomidine for the induction and maintenance of restful sleep.

Specific Aim 1: Investigate the safety profile of dexmedetomidine in a phase I dose finding study.
Hypothesis 1.1. Oral dexmedetomidine will maintain cardiovascular stability
Hypothesis 1.2. Oral dexmedetomidine will result in electroencephalographic evidence of spindle and slow/delta oscillation.

Specific Aim 2: Investigate the efficacy of dexmedetomidine for inducing sleep in a Phase II randomized double-blind placebo-controlled study.
Hypothesis 2.1. Oral dexmedetomidine will reduce sleep latency
Hypothesis 2.2. Oral dexmedetomidine will improve sleep efficiency

III. Volunteer Selection and Enrollment

Only those subjects capable of giving their own consent will be considered for this study.

Phase 1: We will select 15 male and female healthy controls for this study between the ages of 18 and 50.
Phase 2: We will select 15 male and female healthy controls for this study between the ages of 18 and 50.

The volunteers will be recruited using an announcement of the study distributed through the Partners Public Affairs distribution list. All volunteers will be fit and healthy, meeting the American Society of Anesthesiologists (ASA) physical status classification P1 (normal healthy subjects) and P2 (stable chronic condition) and of normal body habitus. Prior to the study enrollment, each volunteer will sign informed consent. A complete medical history will be taken in addition to performing a complete physical examination in order to rule out active and chronic medical problems.

Medical History

Chronic health conditions that will exclude volunteers from the study include but are not limited to:

Cardiovascular:	Poorly controlled hypertension, myocardial infarction, coronary artery disease, peripheral vascular disease, arrhythmia, congestive heart failure, valvular disease, cardiac electrophysiological abnormalities ascertained from the screening ECG (i.e. but not limited to second and third degree heart block in the absence of a pace maker, supraventricular tachycardia, ventricular tachycardia, atrial fibrillation, atrial flutter, junctional heart rhythms)
Respiratory:	Bronchitis, chronic obstructive pulmonary disease, smoking, shortness of breath, history of obstructive sleep apnea
Hepatic:	Hepatitis, jaundice, Child-Pugh Class C liver disease, generalized anasarca, ascites, encephalopathy and caput medusa
Neurologic:	Seizure, stroke, positive neurologic findings on neurologic examination, multiple sclerosis, Meniere's disease, Parkinson's disease

Gastrointestinal:	Esophageal reflux, hiatal hernia, ulcer, oral ulcers, or other oral pathology
Endocrine:	Diabetes, thyroid disease
Hematologic:	Blood dyscrasias, anemia, coagulopathies
Musculoskeletal:	Prior surgery or trauma to head neck or face, arthritis, personal or family history of malignant hyperthermia
Psychiatric:	History or treatment for an active psychiatric problem, including, but not limited to: attention deficit hyperactivity disorder, depression, bipolar disorder, and schizophrenia
Reproductive:	Pregnancy, breast-feeding
Medications:	Regular use of prescription and non-prescription medications expected to affect central nervous system function or sleep
Allergies:	Dexmedetomidine, glycopyrrolate, phenylephrine, ephedrine, labetolol

Physical Examination

The volunteer will be given a standard pre-anesthetic physical examination. Abnormal findings on physical examination will provide reason for exclusion from the study. Abnormal finding(s) will be reported to the affected volunteer and recommendation(s) for medical follow-up will be given as needed.

Screening Tests

A complete blood count, blood glucose level, liver function test (LFT), blood urea nitrogen (BUN), and creatinine (Cr) level will be obtained at the initial screening visit. For inclusion into the study protocol, each volunteer will be required to have a platelet count, blood glucose, BUN, Cr and LFT's levels be within 1.5 times the upper limit of normal. Each volunteer will provide a urine specimen for a toxic substance screen both at the initial examination and on each active study day. Each female volunteer will be administered a pregnancy test at the initial examination and each active study day. Positive toxicity screening and/or positive results on the pregnancy test will prompt exclusion from the study. A 12-lead electrocardiogram will be performed on all study volunteers at the initial screening visit to identify any pre-existing cardiac issues.

Primary Inclusion Criteria for "Healthy" control volunteers

- Age between 18-50
- Native English speaking
- ASA physical status classification P1 and P2 (stable chronic condition)
- Normal body habitus.

Primary Exclusion Criteria for "Healthy" control volunteers

- Abnormal sleep habits
    - Sleeping less than 5 hours each night
    - Going to sleep before 9:00 PM or after 2:00 AM on a regular basis
    - Waking up before 5:00 AM or after 10:00 AM on a regular basis.
- Takes medication that alters sleep, cognitive function, or both.
- Has a history of a known neurological or psychiatric problem.
- Younger than 18 or older than 50 years of age.
- Known or suspected sleep disorder(s).

Remuneration

For successful completion of Phase I of this protocol volunteer remuneration will be $500. If the study volunteer is unable to complete the entire protocol, the proration will be as follows:

- Study volunteers who complete the medical evaluation (screening visit) but do not begin the active portion of the study will receive no remuneration.
- If the study must be stopped due to concerns for the study volunteer's medical safety, volunteers will receive $500.

For successful completion of Phase II of this protocol volunteer remuneration will be $750. If the study volunteer is unable to complete the entire protocol, the proration will be as follows:

- Study volunteers who complete the medical evaluation (screening visit) but do not begin the active portion of the study will receive no remuneration.
- Study volunteers who complete only one sleep study night will receive $100.
- Study volunteers who complete two sleep study nights will receive $300.
- Study volunteer who complete all sleep visits will receive $750.
- If the study must be stopped due to concerns for the study volunteer's medical safety, volunteers will receive remuneration commensurate with their time given to the study as listed above.
- Participants will also be paid a bonus of $0.05 for every correct sequence on the MST task on the three overnight visits.

IV. Study Procedures

An Investigational New Drug (IND) application has been submitted to the Federal Drug Administration (FDA) and study initiation will be contingent upon IRB and FDA approval. Also, we will meet with Emily Ouellette, QI Program Assistant Director, FDA and Regulatory Support to make certain that this protocol is in compliance with all FDA regulations.

All study visits will take place at the White 13 Clinical Research Center, except Phase 1, Visit 2, which will take place at the White 5 at the Carl Rosow Center for Clinical Research. This facility was constructed specifically for the performance of research on human volunteers receiving medications used in anesthesia. The research area is built to the same standard as a typical surgical procedure room at MGH with respect to ventilation, temperature control, plumbing and electrical facilities, as well as storage for sterile supplies and linens. There is piped oxygen and air, wall suction and waste anesthetic gas removal. The hospital's backup generator supports electrical the outlets in clinical research center. There is a fully equipped anesthesia machine (ventilator) with an automated record keeper and standard anesthesia monitors. A fully equipped code cart is also available, and an emergency call system is connected to the Post Anesthesia Recovery Unit. Personnel from the Operating Room pharmacy check the code cart and medications at intervals, and the anesthesia equipment is checked and maintained by the bioengineering department. In summary, the level of physiological monitoring and clinical care that can be delivered in this facility is comparable to an Intensive Care Unit at MGH and far exceeds what is available in the White 13 Clinical Research Center. We deem this high level of care essential for our dose finding Phase I study.

The initial starting doses selected for this Phase I clinical trial are based on clinical and research experience with intravenous dexmedetomidine, our synthesis of the literature on the pharmacodynamics/pharmacokinetics of oral dexmedetomidine, and clinical experience documented in the literature of the intravenous form of dexmedetomidine when it is administered orally. The starting doses are in accordance Food and Drug Administration's guidelines for Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers which states that toxicity should be avoided at the initial clinical dose. However, doses should be chosen that allow reasonably rapid attainment of the Phase I trial objectives (e.g. Assessment of the therapeutics tolerability, pharmacodynamic or pharmacokinetic profile).

Phase 1a – Oral, will follow an open-label, and dose escalation design. The first 5 subjects enrolled in this phase will receive 300 mcg (dose A) of dexmedetomidine in an unblinded fashion, and will be closely monitored for hemodynamic stability. Assuming that dose A does not cause any adverse hemodynamic events, the next 5 subjects enrolled in the study will receive 500 mcg (dose B). Assuming that dose B does not cause any adverse hemodynamic events, the next 5 subjects enrolled in the study will receive 700 mcg (dose C).

Study Design Schematic Phase I

Visit 1	Visit 2
Consent, Physical Examination, Blood Work etc Location: White 13 Clinical Research Center	Dexmedetomidine (doses A, B, and C) Location: White 5 Carl Rosow Center for Clinical Research.

Phase 1: Dose Escalation Study

Visit 1 (Initial Screening)

Prior to the beginning of the study, the study volunteer will sign informed consent. Next, the volunteer will undergo an initial pre-anesthetic exam and toxicology screening to assess exclusionary criteria. A pregnancy test will be administered to female volunteers. An electrocardiogram will be performed on all study volunteers at the initial screening visit to identify any pre-existing cardiac issues. A complete blood count, blood glucose level, LFT, BUN, and Cr level will be obtained. Volunteers will also complete a questionnaire concerning his/her sleep habits. This questionnaire is in clinical use by the Massachusetts General Hospital sleep laboratory.

Visit 2 (Active Study Day)

The study volunteer will arrive at the study site and a toxicology screen will be administered to exclude the use of prohibited substances, and female volunteers will again be required to undergo a pregnancy test. Physiological measures will include up to 256 channels of EEG, a 5 lead electrocardiogram, pulse oximetry, heart rate, end-tidal CO_2, and audio/video data. All of the leads and sensors used are removed easily. Furthermore, blood samples will be obtained for pharmacokinetic/pharmacodynamic modeling of dexmedetomidine at T = 0 (baseline) and at T = 10 minutes, 20 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours and 7 hours after administration.

An intravenous line will be placed and saline locked for the administration of rescue vasoactive medications if necessary. We will also place an arterial-line to enable real time assessment of beat-to-beat variability in the blood pressure. In contrast to a blood pressure cuff, the arterial line will provide for timely notification and intervention of any untoward events. Dexmedetomidine will be administered according to the predetermined dose escalation above. A board certified anesthesiologist will monitor the subject for up to 8 hours after the administration of the medication to ensure normal physiology is maintained. Upon the conclusion of testing a member of the study staff will remove the physiological monitors. Prior to leaving the study site, each volunteer will complete a brief questionnaire. Discharge home will be based on criteria established by the MGH Department of Anesthesia practices for discharge from the hospital following ambulatory surgery. The volunteer must have stable vital signs, i.e., within 20% of pre-study values, be able to respond appropriately to normal commands, be pain free, be free from any nausea and vomiting. Volunteers will be advised not to drive or operate heavy equipment for 24 hours.

The primary endpoint for the Phase I studies will be hemodynamic stability defined as (1) hypotension: systolic blood pressure (SBP) < 60 mmHg, diastolic blood pressure (DBP) < 40 mmHg or decrease of SBP by ≥ 50 % from the baseline which is sustained over two consecutive minutes, (2) hypertension: SBP > 180 mmHg, DBP > 100 mmHg, or increase of SBP by ≥ 50 % from the baseline which is sustained over two minutes and, (3) bradycardia: heart rate (HR) < 40 bpm or decrease by ≥ 50 % from the baseline sustained over two consecutive minutes. Intravenous ephedrine, phenylephrine, glycopyrrolate and/or fluid bolus (500 ml of 0.9% normal saline) may be administered for the treatment of hypotension. Intravenous labetolol may be administered for the treatment of hypertension. The anesthesiologist based on his/her clinical judgment will administer all medications. Standard department guidelines will guide the administration of all ancillary medications. Secondary endpoints include the occurrence of dexmedetomidine-spindle, slow, and delta oscillations on the electroencephalogram. If no adverse events occur during the Phase I trial, we will then request the DSMB to review our results before proceeding with the Phase II trial.

Study Design Schematic Phase 2: Crossover Study

Visit 1	Visit 2	Visit 3	Visit 4
Consent, Physical Examination, Blood Work etc Location: White 12 Clinical Research Center	Acclimation Night Location: White 12 Clinical Research Center	Dexmedetomidine or Placebo Location: White 12 Clinical Research Center	Dexmedetomidine or Placebo Location: White 12 Clinical Research Center

Next, in a Phase II randomized double-blind placebo-controlled crossover study, 15 healthy volunteers will undergo one night of polysomnography acclimation, and two active polysomnography night visits. During the active visits, each volunteer will be randomized in a double-blinded fashion to receive either dexmedetomidine or placebo followed by polysomnography recording of 8 hours. The Phase II dose of dexmedetomidine will be 700mcg oral, as determined by our Data Safety and Monitoring Board. We have concluded from our review of the literature and our proof-of-concept study that the aforementioned doses should be efficacious while maintaining cardiovascular stability. A requirement for this safe dose will be the maintenance of systolic blood pressure and the heart rate within 25% of baseline values without the administration of vasoactive medication. The MGH Research Pharmacy will consult the randomization assignment and will prepare the appropriate treatment for the volunteer. The primary endpoints for the Phase II study are polysomnography measures of sleep latency and efficiency. Secondary endpoints include subjective and objective measures of sleep, memory consolidation and learning.

Phase II

Visit 1 (Initial Screening)

Prior to the beginning of the study, the study volunteer will sign informed consent. Next, the volunteer will undergo an initial pre-anesthetic exam and toxicology screening to assess exclusionary criteria. A pregnancy test will be administered to female volunteers. An electrocardiogram will be performed on all study volunteers at the initial screening visit to identify any pre-existing cardiac issues. A complete blood count, blood glucose level, LFT, BUN, and Cr level will be obtained. Volunteers will also complete a questionnaire concerning his/her sleep habits. This questionnaire is in clinical use by the Massachusetts General Hospital sleep laboratory.

Visit 2 (Acclimation Night)

In this crossover study, the study volunteer will arrive at the study site and a toxicology screen will be administered to exclude the use of prohibited substances, and female volunteers will again be required to undergo a pregnancy test. Physiological measures will include; up to 256 channels of EEG, 2 EOG channels, 2 chin EMG channels, a 5 lead ECG, saturated oxygen levels, heart rate, end-tidal CO_2, and audio/video data. All of the leads and sensors used are removed easily. The EEG cap will be placed on the volunteer to enable acclimation to the device. ECG, EOG and EMG channels will be attached so that the volunteer can acclimate to the sensation of contact at these points. The purpose of the first study night is for the volunteer to become acclimated to the study site and recording equipment, as well as to obtain a baseline reading of their brain activity during sleep. The volunteer will then be instructed to try to sleep for 8-hours. If he/she wakes up before the designated time, he/she will be instructed to stay in bed and try to go back to sleep. A registered nurse will monitor the volunteer all night. An on-call physician is available, at all times, in the event of an unforeseen problem. Prior to leaving the study site, each volunteer will complete a brief post-sleep questionnaire. Upon conclusion of testing, a member of the study staff will remove the physiological monitors and the volunteer will be permitted to resume his/her day as normal. The volunteer must have stable vital signs, i.e., within 20% of pre-study values and be able to respond appropriately to normal commands prior to discharge home. Volunteers will be advised not to drive or operate heavy equipment for 24 hours.

Visit 3 and 4 (Active Study Night)

Due to the crossover nature of this Phase II study, the volunteer will return to the study site on two separate nights to complete study procedures. A toxicology screen will be administered to exclude the use of prohibited

substances. The electrodes on the EEG cap and the ECG, EOG and EMG channels will be attached per standard procedure. Saturated oxygen levels, heart rate, and end-tidal CO_2, will be monitored. No arterial line will be placed in these volunteers. On each of the study nights, dexmedetomidine or placebo will be administered according to the predetermined randomization list by the MGH research pharmacy. However, both dexmedetomidine and placebo will not be administered on the same night. All volunteers will receive both dexmedetomidine and placebo either on Visit 3 or 4. A board certified anesthesiologist will monitor the subject for 1 hour after the administration of the medication to ensure normal physiology is maintained. A registered nurse will monitor the volunteer all night. An on-call physician is available, at all times, in the event of an unforeseen problem. The volunteer will then be instructed to try to sleep for 8-hours. If he/she wakes up before the designated time, he/she will be instructed to stay in bed and try to go back to sleep. Reflecting standard of post procedure monitoring, the study anesthesiologist will monitor the subject for one hour after the administration of the medication to ensure normal physiology. Prior to leaving the study site, each volunteer will complete a brief post-sleep questionnaire. Upon conclusion of testing, a member of the study staff will remove the physiological monitors and the volunteer will be permitted to resume his/her day as normal. The volunteer must have stable vital signs, i.e., within 20% of pre-study values and be able to respond appropriately to normal commands prior to discharge home. Volunteers will be advised not to drive or operate heavy equipment for 24 hours.

Cognitive Testing

Motor Sequence Task: Participants will be trained on a motor sequence task (MST), a well-established probe of sleep-dependent memory consolidation, on a laptop prior to the onset of sleep and then tested 8 hours later. The MST involves pressing four keys with the fingers of the left hand, repeating a five digit sequence (*e.g.*, 4-1-3-2-4) "as quickly and accurately as possible" for 30 seconds. During both the training (night time) and test sessions (morning), participants will perform twelve 30-second tapping trials each of which is followed by a 30 second break. During tapping trials, the computer screen will be green with the numeric sequence displayed at the top, and dots appearing from left to right beneath the sequence with each keystroke. During the breaks, the display will be red, and instead of showing the sequence, numbers (displayed as words) counted down the seconds until the next trial. Three seconds before the display turns green, the words will be replaced by flashing dots to alert the participant. We will measure the number of correct sequences per 30 second tapping trial, which reflects the speed and accuracy of performance. Any unfinished sequence at the end of a trial will be added to the total, as a fraction of a correct sequence. Next-day improvement will be calculated as the percent increase in correct sequences from the last three training trials to the first three test trials. Learning during training will be calculated as the percent increase in correct sequences from the first training trial to the average of the last three training trials. Participants will be paid a bonus of $0.05 for every correct sequence.

Psychomotor Vigilance Task (PVT): is a highly sensitive assay of vigilant attention used commonly in the study of sleep disruption after each night of sleep.

While seated at a laptop computer, the subject will be asked to attend to an area enclosed by a small green rectangle against a black screen. After a variable delay (chosen randomly to last between 2 to 10 seconds) a digital millisecond counter will begin inside the rectangle.
The subject will be asked to stop the timer as quickly as they can by pressing the space bar. At this time, the subject can view his/her reaction time, which serves as feedback for that trial.

Instruction is given to avoid premature button presses during the variable inter-stimulus interval. If such a false start occurs, the subject will be asked to wait for the timer to begin before pressing the space bar and a new trial will commence. Trials of this nature repeat throughout a 10 minute session.

The following instructions will be presented to the subject:

> *Welcome. In this task, a rectangle will appear in the center of the screen. After a delay of variable duration, a counter will begin inside this rectangle. Your job is to press the SPACEBAR as rapidly as possible once this timer begins. Hitting the spacebar will stop the timer; you will then have a chance to view your speed. Aim for low numbers, but be sure not to press the spacebar before the timer starts! The task will continue for 10 minutes, and the computer will let you know when you have finished.*

From this task we will analyze outcome measures such as the median reaction time, the variability of these reaction times, the occurrence of "lapses," (response times over 500 ms) errors of commission ("false-starts"), and a measure of performance degradation through time. The subject will perform this test before and after sleep on each study day.

V. Biostatistical analysis

Using an endpoint of sleep latency to guide sample size calculations. Our null hypothesis is the average within volunteer difference in sleep latency for oral dexmedetomidine compared with placebo is zero. Alternative hypothesis is the average within volunteer difference in total sleep time for dexmedetomidine compared with zolpidem is 30 minutes. Assuming a standard deviation of 10 minutes in the dexmedetomidine groups and 15 minutes in the placebo group, a type I error of 0.05 and minimum power of 0.80, the sample size determinations for our Phase II study is 4. Therefore, we should detect a clinically relevant improvement in sleep latency if we successfully enroll only 5 volunteers for the each of the dexmedetomidine groups.

VI. Risk and Discomfort

Dexmedetomidine: The risks involved in the administration of dexmedetomidine include nausea, xerostomia, atrial fibrillation, and transient hypertension during drug loading. The significant risks involved are directly related to a drug induced reduction in sympathetic activity, resulting in hypotension and bradycardia. Rare case reports of sinus arrest in instances of rapid drug administration and in subjects with a high resting vagal tone have also been described. Drug discontinuation, dose reduction, or the use of vasoactive substances causes a return of these hemodynamic parameters to baseline. As such, study volunteer hemodynamic parameters will be continuously monitored to ensure that appropriate medical intervention will be instituted for any clinically significant hypotensive or bradycardic episodes.

Polysomnography risks: Electrodes placed on the scalp may cause temporary redness.

Intravenous line risks: Pain at the needle insertion site, infection, and bleeding.

Arterial Line risks: The most concerning risk is a <0.01% of permanent ischemic injury with placement of an arterial catheter in the radial artery. The risk factors that have been identified in the medical literature include high severity of illness (American Society of Anesthesiologists IV and greater), low cardiac output, use of vasopressor therapy, prolonged duration of catheter placement (greater than 4 days), large catheter size, multiple punctures for catheter insertion, predisposing comorbidities (i.e. peripheral vascular disease), diabetes. The study volunteers we will enroll are healthy ASA I/II and by definition are without any of these comorbid conditions. Our research group has successfully executed three research protocols (2005P001549, 1999P010748, and 2011P002333) that incorporate the use of arterial lines to monitor subjects being studied under general anesthetics. Under the aegis of these protocols, arterial lines have been placed without any ischemia related tissue injury. Other risks include pain at the needle insertion site, infection, bleeding, AND arterial thrombosis (blood clots).

VII. Potential Benefits

There are no direct benefits to the individual volunteers involved in this study. The potential benefits of this study to society are a clearer understanding of the potential benefit of dexmedetomidine as a therapy for insomnia compared with the established therapy zolpidem. We hope to also gain insight into the extent of which dexmedetomidine affects the sleep architecture of healthy volunteers. If successful, this study may lead to the identification of a new class of medications for sleep therapy.

VIII. Monitoring and Quality Assurance

All EEG, blood pressure, heart rate, electrocardiography, and capnography data will be visualized in real time for safe monitoring. These data will also be stored for later off-line analysis. Unanticipated problems involving risks to volunteers or others, including adverse events will be reported to the PHRC in accordance with PHRC unanticipated problems including adverse events reporting guidelines.

Data and Safety Monitoring Board (DSMB)

A Data and Safety Monitoring Board (DSMB) has been created as an independent body charged with ensuring that the safety of study volunteers is protected and that the scientific goals of the study are being met. To

support those purposes, the DSMB will perform expedited monitoring of all serious adverse events, perform ongoing monitoring of drop-outs and non-serious adverse events, determine whether study procedures should be changed or the study should be halted for reasons related to the safety of study volunteers, and perform periodic review of the completeness and validity of data to be used for analysis of safety and efficacy. The DSMB will also ensure volunteer privacy and research data confidentiality.

Serious Adverse Events

Expedited review will occur for all events meeting the FDA definition of SAEs – i.e., any fatal event, immediately life-threatening event, permanently or substantially disabling event, event requiring or prolonging inpatient hospitalization. This also includes any event that a study investigator or the DSMB judges to impose a significant hazard, contraindication, side effect, or precaution. For purposes of this study, all SAEs are required to be reported to the DSMB, regardless of any judgment of their relatedness to the study drug. All relevant information will be reported to the DSMB for each SAE including information about the event and its outcome, dosing history of all study drugs, concomitant medications, the volunteer's medical history and current conditions, and all relevant laboratory data. Notification by e-mail of all related study forms shall be made to the DSMB within 2 days of the occurrence of any SAE. Information will be reviewed and a determination made of whether there was any possible relevance to the study drug. Additional reporting to the IRB will be done within 24 hours of the SAE.

Other Safety-Related Reports

At twelve-month intervals throughout the course of the study, the DSMB will also receive unblinded summary reports of treatment retention and reasons for dropout, by treatment arm and study phase.

Study Stopping Rules

If at any time during the course of the study, the DSMB judges that risk to volunteers outweighs the potential benefits, the DSMB shall have the discretion and responsibility to recommend that the study be terminated.

Monitoring of Data Quality by the DSMB

At least on a yearly basis during the course of the study, the DSMB will receive a report on data quality and completeness. The Charter and Membership has been submitted to the IRB.

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