UNderstanding CONSciousness Connectedness and Intraoperative Unresponsiveness Study (UN-ConsCIOUS)

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UN-CONSCIOUS Protocol

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Version Date: 1/11/2023

Investigator's Agreement

1. I have read this protocol and agree to conduct this trial in accordance with Good Clinical Practice (GCP), all stipulations of the protocol, the Declaration of Helsinki, and applicable regulatory requirements as stated by my human subjects testing oversight body [e.g., independent ethics committee (IEC) or institutional review board (IRB)].

2. I will personally conduct or supervise the described investigation(s). This includes informing all associates, colleagues, and employees assisting in the conduct of the study about their obligations in meeting the above commitments.

3. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

4. I agree that all electronic signatures will be considered the equivalent of a handwritten signature and will be legally binding.

Protocol Title: UNderstanding CONSciousness Connectedness and Intraoperative Unresponsiveness Study (UN-CONSCIOUS)

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Signature of Principal Investigator | Date

Name of Principal Investigator (printed or typed)

Summary

The aim of the proposed research is to measure the effect of drug-induced sedation on delta power (also known as slow wave activity) of posterior cortex (overlying the precuneus brain region) and the functional and effective connectivity between brain regions as indicated by high density EEG transmission within the human brain. The results of this study will help to identify the neurophysiological correlates of changes in conscious experience that occur during drug-induced sedation informing clinical practice of sedation and anesthesiology as well as consciousness science.

The study will employ auditory stimuli and high-density electroencephalography (HD-EEG) in combination. We hypothesize that consciousness during sedation will be associated with lower delta power at the CPz electrode than unconsciousness. HD-EEG allows non-invasive recording and mapping of brain responses. Transmission of signals within the brain will be evaluated in normal subjects by recording the time course and spatial distribution auditory stimuli-evoked EEG signals during different states of sedation i.e., wakefulness, responsive sedation, and unresponsive sedation. Sedation will be induced with FDA-approved, clinical sedatives, dexmedetomidine, ketamine, midazolam and propofol. All these drugs have distinct mechanisms of action. We will study 20 patients for each drug. Hence 60 subjects will be recruited in total. We will compare these responses from those obtained from natural sleep with sleep data acquired at the Wisconsin Psychiatric Institute and Clinics (WISPIC).

We have previously shown that midazolam, a short-acting benozodiazepine that is commonly used for procedural sedation and before and during surgery is safe. Herein we seek to develop these same findings throughout the study of the other sedative drugs. In the proposed doses, there is a very small risk of prolonged sedation and loss of airway control. All drugs will be administered by an anesthesiologist with clinical grade equipment and facilities. The sedative drugs (dexmedetomidine, ketamine, midazolam and propofol) have very low allergic potential.

Schedule of activities

	Screening	Baseline	Sedation
Window	Up to 30 days prior	Up to 30 days prior	
INFORMED CONSENT	X	X	X
ELIGIBILITY REVIEW	X		X
OBTAIN HISTORY & DEMOGRAPHICS	X		X
VITAL SIGN MEASUREMENT	X		X
ANESTHESIOLOGIST REVIEW[1]	X		X
REVIEW OF CONCOMITANT MEDICATIONS	X		X
HD-EEG		X	X
Predictive Coding and Cognitive tasks		X	X
Auditory Stimuli		X	X
Sleep Study		X	
Brice Questionnaire			X
Blood Collection[2]			X
SEDATION[3]			X
Patient Reimbursement	none	$50 for sleep study	$200 for each sedation visit

[1] History and Physical, Neurologic and Anesthesiologic examinations for candidacy will be performed.
[2] Blood samples will be taken intraoperatively from the indicated intravenous line.
[3] Sedation will be with dexmedetomidine, ketamine, midazolam or propofol. We will recruit 20 subjects per sedative. All subjects will have the option to complete all sedation tests. Each sedation will be at least 28 days apart.
*MRI might be performed on a subsequent day, depending on the availability of the MRI machines.

Introduction

1.1. Background

'Consciousness' is synonymous with 'subjective experience': it can be summarized as "what abandons us every night when we fall into dreamless sleep and returns the next morning when we wake up or when we dream." Similarly, patients typically report the absence of subjective experience upon recovering from anesthesia (though they sometimes also report dreams). It is evident that sleep and anesthesia are not the same. But, they do share certain behavioral features, such as unresponsiveness and loss of muscular tone. They also share a number of neurophysiological characteristics, including EEG slowing and synchronization, characteristic events such as "K-complexes" and "spindles", and partially overlapping patterns of subcortical activity patterns revealed by microelectrode and imaging methods. Although there are similarities, there are also several notable differences between sleep and anesthesia. For example, whereas sleep may be interrupted by a noxious stimulus or even a loud noise, anesthesia – at clinical doses - survives these challenges. Does this mean that the unconscious state produced by sleep and anesthesia are fundamentally different? This is one of the fundamental questions we will address.

Anesthetics appear to produce a reversible state of unconsciousness accompanied by amnesia[1]. This remarkable phenomenon brings great relief to surgical patients and wonder to clinicians and scientists. However consciousness also occurs during anesthesia: dreaming is common under clinical conditions (33% of patients) and in laboratory studies (up to 80% of subjects[2]) but subjects typically remain "disconnected" from their environment (ie. they may be in a dream-state, unaware of their environment and the external sensory world)[1]. This complex pharmacological state mirrors that of sleep but to date, we do not fully understand the mechanisms by which anesthetics ablate conscious sensation and memory. Nor do we have a deep understanding of consciousness itself or how to measure it – a shortcoming that hinders progress in the newly emerging science of consciousness, and has prevented the development of an effective diagnostic tool or monitor that can be applied in clinical settings.

The intense interest in "depth-of-anesthesia" monitors demonstrates its perceived importance among practicing clinicians. Just as monitors of cardio-respiratory function dramatically improved patient safety, a reliable monitor of consciousness has the potential to reduce the incidence of awareness with recall, which remains unacceptably high (up to 0.2%) and can lead to adverse psychological sequelae, such as symptoms of post-traumatic stress disorder. At the same time, there is a concern that many patients may be "overdosed" in an attempt to minimize awareness with recall.

Current approaches to measuring depth-of-anesthesia suffer from significant inter-patient variability in response properties. They also lack predictive power: although valid on average, they fail too often to report the level of consciousness in individual patients[1]. We suggest that an approach based on a fundamental understanding of consciousness is an essential path to identifying a universally applicable measure that can be used to guide drug administration in the perioperative setting.

Understanding how we may be conscious under anesthesia but unaware of our environment (sensory disconnection) is also critical[1]. Sanders et al. have discussed, achieving sensory disconnection appears desirable in anesthesia but may fail in approximately 37% of cases[1]. Our ConsCIOUS study (IRB: Bauer (PI) 2014: 1558) has shown that 4.6% of patients are aware of their environment following intubation under general anesthesia. Presently we lack a marker of disconnection that provides confidence that sensory stimuli will not trigger a conscious experience. Such a marker would have clinical utility to identify patients at risk of anesthesia awareness. As discussed above, dreaming (disconnected

consciousness) is common under clinical anesthesia. A depth of anesthesia monitor that detected internally-triggered dreaming (with sensory disconnection from surgery) would signal to the anesthesiologist to unnecessarily deepen the anesthetic state increasing the risk of the side effects of anesthesia. A marker of disconnection would have significant clinical utility to signify the lack of perception of surgery. Any insights afforded into the mechanisms of disconnection could lead to developments of improved anesthesia or sedation in the critical care unit as well as development of further sleep aids for use in the community.

Patterns of brain activity during anesthesia can be recorded using electroencephalography (EEG). EEG reflects the electrical activity of the brain, and is characterized by fundamental oscillatory patterns, including slow waves and spindles. A more complete understanding of these patterns will elucidate both normal brain function, and alterations in these patterns may be sensitive indicators of disrupted brain function in disorders of brain function. This aim will be supported by studying four clinical drugs with diverse mechanisms of action: dexmedetomidine (an alpha2 adrenergic agonist), propofol and midazolam (GABAmimetics), and ketamine (NMDA antagonist). While midazolam and propofol both affect GABAA receptor function, midazolam targets synaptic (benzodiazepine sensitive channels) while propofol also targets extrasynaptic channels (and HCN-1 channels in addition). These differences necessitate individual studies of those drugs. We will study the EEG correlates of conscious experience and lack of conscious experience under sedation. In order to obtain report of consciousness, we will wake patients from the sedated state and ask them questions to gain insights into the experience – if any – they were having at the time. We will compare the responses obtained during sedation with those collected during natural sleep. We will test whether EEG responses during sleep correlate with changes during sedation. In the future we envision that sleep data can be used to develop personalized thresholds for an EEG consciousness monitor to be used during sedation and anesthesia and we will test this proposal in a subsequent application based on the data acquired herein.

We will also test the cognitive state when aroused from sedation that we expect will be associated with impaired feedback processing in the brain and therefore disturbed predictive coding[3]. We have recently shown that feedback connections are particularly susceptible to sub-anesthetic concentrations of drugs[4] and we will therefore test whether cognitive processes that depend on feedback processing, particularly those dependent on predictive coding[3], are affected during sedation. We will then titrate the anesthetic to a state of unresponsiveness to verbal command and repeat the experiment (but not the predictive coding tasks requiring the subject to respond).

Our laboratory has pioneered such approaches, particularly in advancing the use of high-density EEG (HD-EEG) in the study of sleep, especially in combination with transcranial magnetic stimulation – a non-invasive method for stimulating specific brain regions and auditory stimuli (IRB: Tononi (PI) 2013-0019). We have successfully and safely used this technology during HD-EEG during sleep and anesthesia[5] (IRB: Pearce (PI) 2008-0018). The combination of HD-EEG, and auditory stimuli, will provide novel insights into EEG and connectivity changes in the brain under varying depths of anesthesia.

1.2. Rationale and hypothesis

This study will be a single-site, controlled, unblinded study at the University of Wisconsin to examine changes in posterior cortical delta power of the HD-EEG during anesthesia and waking. We hypothesize that sedation, with different agents, will increase posterior cortical delta power, interrupting the conscious experience, testing this using several anesthetic drugs.

We will apply high-density electroencephalography (HD-EEG) to assess waking and anesthetized brain activity in response to non-invasive brain stimulation techniques, such as auditory stimulation.

2. Objectives

2.1. Primary outcome and endpoint (endpoint bulleted below)

The study primary outcome is the difference in spontaneous EEG delta power over posterior cortex between consciousness and unconsciousness during sedation.

2.2. Secondary outcomes and endpoints (endpoints bulleted below)

1. The incidence of disconnected conscious experience (dreaming) versus connected conscious experience (awareness of the external world) during sedation.
2. The ability to identify shapes/images in visual illusions and match sounds and images during sedation.
3. The ability to hear words and form implicit memory during sedation tested using a two-alternative forced choice word task.

2.3. Exploratory outcomes and endpoints (endpoints bulleted below)

1. Difference in HD-EEG responses assessed by calculation of the Phase Locking Factor following auditory stimulation between conscious experience under sedation and lack of conscious experience. Phase locking factor is calculated by taking the Hilbert transform of the evoked response and quantifying the alignment of phases based on the imaginery component of the signal. PLF is quantified on a scale of 0 to 1. With 0 being no phase locking and 1 being maximal phase locking. We expect than under anesthesia PLF will decrease across the whole scalp.
2. Drug effect on percent difference in duration of PLF between the two consciousness states.
3. The effect of consciousness during sedation vs. unconsciousness during sedation on PLF following auditory stimulation.
4. The correlation between sedation EEG responses and those from natural sleep.

3. Study design

This study will be a single-site, controlled, unblinded study at the University of Wisconsin to examine changes in delta power during consciousness vs unconsciousness during sedation. We will collect data in wakefulness, light sedation and deep sedation. The primary outcome focuses on light sedation during which data will be collected divided on conscious experience present/absent.

We will study four different sedatives that have different mechanisms of action, to provide a cross section of the drugs used in clinical practice and provide greater insight into the mechanisms through which consciousness may be perturbed. We will recruit 20 subjects per drug into this study. Subjects will not be randomized between sedatives as it is important to start with dexmedetomidine which is the drug that will be easiest to titrate to consciousness/unconsciousness. Drugs will be administered, on separate days, in the order of dexmedetomidine, ketamine, midazolam and propofol.

4. Study population

Patients recruited will be 18-40 years old without contraindication to anesthesia or allergy to study drug. We will recruit 20 subjects per drug (up to 80 total subjects). Subjects will be allowed to be participate in all four sedative experiments.

4.1. Inclusion criteria

- Adults, ages ≥ 18 and ≤ 40 years old
- In good health, determined by the PI on the basis of medical history and a standard assessment for anesthesia to be documented as part of the study record

4.2. Exclusion criteria

- Adults <18 years old or >40 years old
- Pregnancy confirmed on pregnancy test on day of sedation
- Contraindication to anesthesia or allergy to study drug
- Difficult anesthesia: American Society of Anesthesiologists Physical Status greater than 1, per the discretion of the PI. Examples of ASA status include, but are not limited to:
    - Any systemic disease present, such as diabetes, cardiac, pulmonary, or other acute or chronic disorder, or history of smoking
    - Narrow angle glaucoma
    - Abnormal airway examination
    - Any abnormality on medical history and physical examination
    - Snoring or sleep disorders including apnea
    - Antecedent pulmonary aspiration risk (e.g., history GI reflux, heartburn, hiatal hernia)
    - Adverse reaction or allergy with anesthesia or other sedatives
    - Chronic medication use
    - History of difficult anesthesia, laryngoscopy or intubation
    - Family history of difficulty with anesthesia or sedation
    - History of vertigo, nausea or vomiting after anesthesia
- BMI > 35
- Contraindication to HD-EEG for relative parts of the procedures.
- Exclusion from Dexmedetomidine:
    - Resting heart Rate<**60** bpm
    - Known dexmedetomidine allergy
- Exclusion from Propofol:
    - Reported egg or propofol allergy
    - Known ketamine allergy
- Exclusion from Ketamine:
    - History of post-operative nausea and vomiting
    - History of motion sickness
- Exclusion from midazolam
    - Known midazolam allergy

<u>Additional exclusion criteria on the day of sedation:</u>

- Anything to eat or drink for the preceding 8 hours
- Any use of over-the-counter or recreational drugs (including alcohol or tobacco) within the preceding 24 hours
- Any use of sedative or sleep agents within the preceding 24 hours
- Recent change in health, including cough, cold, or fever
- Exposure to anesthesia or sedation in the last 6 days

4.3. Protected populations

Prisoners

Due to the complexity of state and federal requirements governing the participation of prisoners in research, patients who are prisoners will not be considered for participation in this trial.

5. Trial interventions

None of the procedures in this study are considered to be standard treatments. Some of the equipment used in study procedures has been evaluated and approved by the FDA for other applications for other (clinical) applications. As all procedures are considered "research", they do not require specific approval by the FDA Devices Panel, as long as subjects are informed of the nature of the study, and all devices are clearly labeled "WARNING - For Investigational Use". However, most of the equipment used in this study has an FDA approval for clinical use AND/OR an FDA 510(k) pre-market clearance. A summary of FDA approvals of equipment is detailed below:

Electroencephalography equipment: Most EEG equipment has been "grandfathered" by the FDA, as it has been used in clinical settings prior to the 1976 Medical Device Amendment to the U.S. Food and Drug Law. EEG devices in current use are, for the most part, substantially equivalent and maintain this exemption.

High-density (HD) EEG equipment is identical in concept to previously existing EEG equipment. However, HD-EEG has many more individual electrodes placed across the scalp to allow superior spatial and temporal resolution in the analysis of the EEG signals. Analysis of HD-EEG data has become practical only with the advent and improvement of powerful personal computers. High-density EEG systems have been in use since at least the early 1990's, and do not require FDA approval for research use. However, more recently some HD-EEG systems (electrode caps, amplifiers, recording software) have received FDA 510(k) pre-market approval, as these companies intend to market their products for commercial clinical use. In addition to HD-EEG systems, multiple sensor systems used for clinical polysomnography (integrated systems incorporating EEG, respiratory monitoring, movement monitoring, etc. along with amplifier and recording/analysis software) also have 510(k) clearance.

The following systems have FDA 510(k) approval:
- Electrical Geodesics Inc., hydrocel geodesic sensor net (HCGSN) hd-EEG system (64-, 128-, and 256-electrode systems). (http://www.egi.com)
- Philips-Respironics Alice Diagnostic Sleep system (http://www.healthcare.philips.com/main/homehealth/sleep/alice5/default.wpd - current vendor for Wisconsin Sleep Center although other systems from Nihon-Koden, Compumedics, ViaSys, etc. may potentially be used in the future)

The following systems do not have FDA 510(k) approval:
- BrainAmp, BrainAmp MR Plus MRI compatible high density EEG system. (http://www.brainproducts.com/products/brainampmrplus/).
- NexStim Eximia EEG system (http://www.nexstim.com/index.php?k=11139)

These systems are designed and marketed exclusively for research applications, and as such do not require FDA approval. However, these devices are not substantially different than the other EEG systems, and are still considered NSR devices. Wireless EEG systems may also be used. These systems consists of battery powered EEG amplifiers that are not directly plugged into the power mains. Several

wireless EEG devices are commercially available. The wireless EEG device currently used for research in the laboratory and approved for use in protocol H2009-0052 was specifically designed for sleep according to ANSI/AAMI ES1-1985 guidelines for "Safe current limits for electro-medical apparatus" and safety standards for wireless communications according to the Radio and Telecommunications Terminal Equipment directives by Philips Applied Technologies.

Auditory Stimulation: Devices that produce evoked auditory stimuli (an EEG response from an auditory stimulus) are described in 21CFR882.1900 as a Class II device (exempt from pre-market notification [510(k) clearance). Auditory stimuli will be produced on a standard personal computer through commonly available media software (e.g. – Windows Media Player, iTunes, etc.) and played back through standard commercially available small speakers or earphones connected to the computer. Volume of playback will be estimated before experiments using a decibel meter, and are maintained below 80dB ("low" volume range, corresponds to a speaking voice level). Volume will kept at the lowest level that still produces an auditory evoked potential (an EEG signal in response to hearing a sound). It is critical to keep the volume as low as possible as louder sounds could awaken a subject and disrupt the experiment.

GE Clinical anesthesia machine: monitoring and full resuscitation equipment consistent with American Society of Anesthesiologists and UW-Madison guidance with FDA approval. Studies conducted in the MRI will have appropriate MRI approved clinical safety equipment including anesthesia apparatus and appropriate staff to safely conduct anesthesia.

EZstim II nerve stimulator, model es400, Life Tech has 510k clearance to provide tetanic stimuli to assess neuromuscular function and is routinely used in clinical anesthesia.

Predictive coding task. We have designed a behavioral task in which subjects use prior auditory information to process visual information. This task uses biomorphic visual stimuli from Michael Tarr's lab, known as greebles (http://wiki.cnbc.cmu.edu/Novel_Objects). Greebles will either be colored or gray-scale images. Each greeble is personified with a name. For example, the violet, green and yellow greebles shown in figure 1 are called Paluti, Kagotu and Tilado respectively. During the experimental session, the subject will first learn two or three pairs of name and image associations through trial and error (images and their names are randomly assigned each session). This phase is called the learning phase, and it will take place before the administration of anesthetic agents. Subjects will hear a name voiced by the computer followed by a visual image of a greeble on the monitor. Auditory feedback on correct and incorrect responses provided at the end of each trial. To confirm that subjects have learnt the correct associations, subjects perform a match/non-match task (figure 2A). Subjects report whether the auditory-visual pairs matched or not using a button response device (keyboard, mouse or button box). The buttons signaling a match and non-match on each trial are respectively indicated by a star (or check) and null (or cross) symbol on the monitor (after the presentation of the greeble image). The appropriate button changes randomly over trials to minimize motor preparation (on half the trials, the left button will signal a match, and on the other half, the right button will signal a match). To allow measuring of reaction times, we will also use a variant of the task shown in figure 2, in which the second delay period (after the greeble image) is absent.

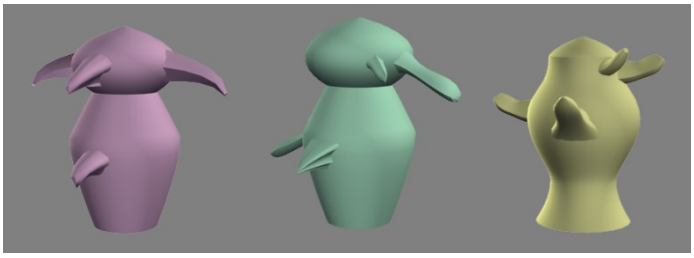
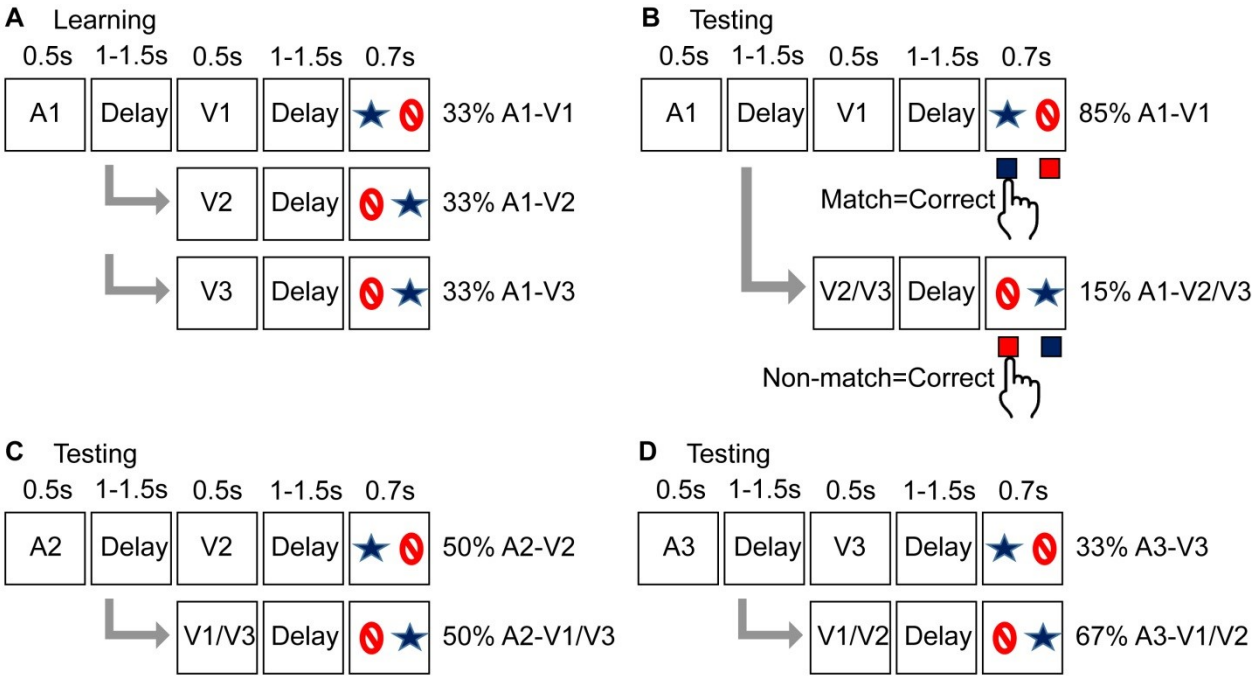


Figure 1 (left). Stimuli used in the behavioral task. To avoid differences in salience of stimuli, greeble images have similar size, number of extensions and contrast, and greeble names have the same number of syllables, start with a 'hard' consonant and end with a vowel.

Figure 2 (below). Task design. A1, A2 and A3 are computer-voiced names Paluti, Kagotu and Tilado. V1, V2 and V3 are greeble images. Star=match; null sign =non-match; screen position indicates left/right button.



Once subjects demonstrate greater than 80% accuracy in the match/non-match task during the learning phase, they progress to the testing phase (figure 2B-D). In this phase, which will occur during baseline recordings, the administration of anesthetic agents (prior to loss of consciousness), and during recovery we manipulate predictions by changing the probability of a greeble visual image appearing after its associated auditory name. For example, whenever subjects hear the name Paluti, there will be an 85% chance of Paluti's image being shown; whenever subjects hear the name Kagotu, there will be a 50% chance of Kagotu's image being shown; and whenever subjects hear the name Tilado, there will be a 33% chance of Tilado's image being shown. In an individual experimental session, there will be roughly equal numbers of match and non-match trials, to avoid response bias. In addition, there will be approximately equal numbers of Paluti, Kagotu and Tilado images, and their corresponding names will have similar probability of being presented as the auditory cue, to control for stimulus familiarity.

To test how precision (or the reliability of sensory information) is represented, in some cases, we may introduce two conditions during the predictive coding testing phase. These are high noise and low noise conditions. White noise will be added to the greeble images to manipulate perceptual confidence.

To rule out the possibility that expectation of a match or non-match in itself may contribute to subjects' responses, we will pilot a variant of the predictive coding task in which half of trials subjects simply respond to the color of the visual image (or, in the case of gray-scale images, whether the image is upright

or inverted) rather than whether the auditory name matched the subsequent greeble image. In this behavioral pilot, the second delay period (after the greeble image) is absent, to allow measuring of reaction times. Further, on the half of trials in which subjects simply respond to the color of the visual image (or, in the case of gray-scale images, whether the image is upright or inverted), a colored swatch (or, for gray-scale images, and up/down arrow) on each side of the fixation point replace the star and null symbols at the end of the trial (see figure 3). Our pilot behavioral data suggests expectation of a match or non-match in itself contribute little to subjects' responses.

Most of this experimental phase is conducted prior to sedation commencing with the testing phase that will take approximately 20 minutes occurring under sedation after waking to answer commands. The testing will be spread over the first 2-3 epochs of arousal after the questions have been asked (equivalent to OAAS scale 4-5) and then the subject will be allowed to return to sleep. The duration of sedation will not be extended for this experimental addition. Patients will be asked to perform the 20 minute testing phase again after full recovery from the drug. This would allow us to look at cognitive performance following recovery from anesthesia.

This task requires active involvement of the subject but as they get more sedated this is not possible. Therefore we will also apply a passive tasks as a pilot study that involves passive audio-visual integration where in addition to a tone, a paired visual stimulus is provided (for example a picture of a face is provided) on the computer screen. Each tone and/or face is provided as a 250ms stimulus. The tone will be provided through the headphones as above and the face will be displayed on a computer screen as per [6]. Fifteen minutes of data will be acquired using this paradigm under sedation. The paired visual and auditory stimulus produces an integrated evoked response [AV]. The interaction of the responses can be calculated by the difference between the AV responses and the visual (V) or auditory cue (A) i.e. (AV – (A+V)) which we call the AV interaction. We will model how sedation affects the AV interaction response hypothesizing that during states of disconnection or unconsciousness the AV interaction will disappear.

Cognitive Tests: To better understand what areas of cognition are affected by anesthesia, participants may also complete a flanker task, verbal fluency task, and Wisconsin card-sorting task. Participants would complete these tasks during the HD-EEG recording. To obtain baseline cognition, these tasks will be completed before the start of sedation. They will also be completed during the lighter sedation period. Similar to the Predictive Coding task, once participants reach a deeper level of sedation, they will be unable to perform these tasks so they will only be done when the patient is still conscious. Subjects will be asked to complete them again during recovery in the CRU, taking approximately 30 minutes. If the subject would prefer this recovery cognitive testing may also be deferred to the following day. The time to complete these tasks will be included in the 4 hours of sedation and the 30 minutes allotted in the CRU. Sedation time will not be increased for these tasks.

The flanker task is a test of attention, executive control, and inhibition. For this task, participants are instructed to focus on a central, directional, target stimulus such as an arrow. The target stimulus will be

surrounded by non-target stimuli. The non-target stimuli will either have congruent or non-congruent directionality with the target stimulus. The participant will be told to respond with the direction of the target stimulus and their accuracy and reaction time will be recorded. Participants respond more quickly when the flanking symbols are congruent with the central target.

For the verbal fluency task, participants will be told to think of words starting with a certain letter. They will be presented with the letters in four 20-s blocks with 20-s blocks of rest. The letters they will be presented with are "F," "A," "S," and "T".

The Wisconsin Card Sorting Task tests executive function and cognitive flexibility. For this task images are presented that vary along different dimensions (e.g., shape and color). Participants are then instructed to sort the images along one of the dimensions. During switch trials, the dimension in which the participant is asked to sort changes. Their ability to quickly and accurately switch between dimensions is considered a measure of cognitive flexibility.

Auditory Stimuli:

Oddball Paradigm:
We will use the "roving" oddball paradigm that our collaborator, Dr. Boly (UW-Madison, Neurology) used previously to study brain-damaged patients with altered levels of consciousness[7] (Fig. 1) and that Dr. Tononi (UW-Madison, Psychiatry) is investigating in sleeping subjects. This paradigm has been well studied in other volunteer settings but not specifically in anesthesia. The stimuli comprise a structured sequence of pure sinusoidal tones. The loudness of the tones will be set in each subject to a comfortable level, which will be maintained throughout the experiment. Within each stimulus train, all tones are of one frequency and are followed by a train of a different frequency. The first tone of a train is a deviant, which becomes a standard after several repetitions. This paradigm ensures that deviant tones and standard tones have exactly the same physical properties, differing only in the number of times they have been presented, which is varied pseudo-randomly between one and eleven. The frequency of the tones will vary from 500 to 800 Hz, in randomly selected integer multiples of 50 Hz. Stimuli will be presented binaurally via headphones for 20 minutes during wakefulness. The duration of each tone will be 70 ms and the inter-stimulus interval is 500 ms. 200 "standard sets" ranging from 1-11 tones/set (also resulting in 300 deviant tones) will be presented to each subject in each condition.

Local/Global Deviant Paradigm:
Experimental stimuli are vowels /a/ and /i/, presented in a local global deviant paradigm (Bekinschtein et al., 2009[8]; Fig. 1). The vowels were excised from the steady-state vowel portions of consonant-vowel stimuli /had/ and /hid/, spoken by a female talker (F_0 = 232 Hz and 233 Hz, respectively) (Hillenbrand et al., 1995). On each trial, five 100 ms vowels, normalized to the same root-mean-square amplitude, gated with 5 ms on/off ramps and separated by 50 ms silent intervals, will be presented, with the fifth vowel being either the same as the first four or different (Fig. 1a). This difference constituted the local deviance: /aaaaa/ and /iiiii/ trials were local standards, while /aaaai/ and /iiiia/ trials were local deviants.

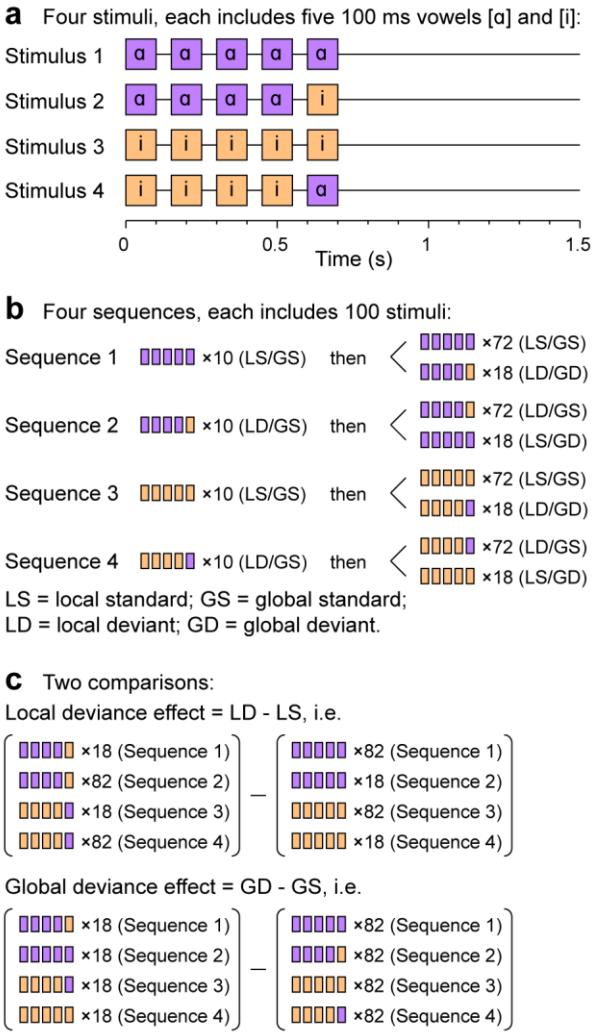


Figure 1. Local global deviant experimental paradigm. a: Schematic of the four experimental stimuli. b: Stimulus sequences. c: Comparisons between trials to characterize local and global deviance effect.

The intertrial interval will be varied within a Gaussian distribution (onset-to-onset mean 1500 ms, S.D. = 10 ms) to reduce heterodyning in the recordings secondary to power line noise. In each block, the stimuli were presented in four sequences, with the order of the sequences randomized across blocks (Fig. 1b). Each sequence will begin with a recorded instruction that defined the task and the target stimulus to the subject, e.g., for Sequence 1: “This time, press the button when you hear this sound: /aaaai/. Once again, press the button when you hear this sound: /aaaai/.” This will be followed by a sequence of 10 trials that established the global standard stimulus, and then by 72 global standard trials and 18 global deviant trials, presented in a pseudorandom order. The difference in presentation frequency thus constituted the global deviance, and the identity of the global deviant changed across the four sequences within each block. The duration of each block is 11 minutes (400 1.5 s trials and four 15-second instruction segments); this will be paused during wake ups. Stimuli will be delivered to both ears via insert earphones that were integrated into custom-fit earmolds. Acoustic stimulation will be performed at a comfortable level, typically 60-65 dB SPL. Subjects will be instructed to report detection of the target stimuli (i.e. global deviants) by a button press if they are awake.

Word Retrieval Task

At one arousal, subjects will be presented with the following words and then, on recovery from sedation in the CRU, they will be tested for memory of the words using a word retrieval task (analogous to ConsCIOUS2). Subjects will be randomized to one of two word list sequences:

List A:

BIRDS
NORTH
TABLE
HAT
PENCIL
LAMP
FISH
SEA
STOVE
STONES
WATER
BASKET
INDUSTRY
OFFICE
EGG
JOURNAL

List B:

BEARS
SOUTH
STREET
TRUCK
BUTTER
BOWL
SHEEP
WOOL
CLOCK
TREES
GLASS
VIOLIN
MATERIAL
HISTORY
FORK
ENGINE

Paradigm:

For encoding, subjects will be presented 16 target words read from either List A or B.

Memory will be assessed and analyzed using the two-alternative forced choice (2AFC) signal detection method in the CRU. Here, the subjects are presented with a pair of words; one is a target word, and the other is a foil. The subjects are asked to select the word that seems more familiar, or otherwise to choose randomly. The following methods are applied to minimize sources of bias: (1) 2AFC word pairs are balanced for linguistic characteristics of valence, arousal, dominance, frequency, and syllables[1] [2], and avoidance of semantic or phonemic similarity across pairs; (2) Alternate versions of the command sequence are created to balance the word used as the target and that as the foil, and applied randomly; (3) To avoid position effects in the 2AFC pairing, alternate versions of the retrieval list are created, and applied randomly.

Limitations:
The 2AFC method is a sensitive, but not specific assessment of implicit memory. Any explicit memory trace will also influence the discrimination of target words. This limitation is acceptable because: (1) There is a sound a priori hypothesis that explicit memory will be absent or rare during sedation/anesthesia; (2) In the context of anesthetic patients who report no spontaneous recollection, the existence of any memory behavior that represents performance above chance is of interest and significance; (3) Alternate methods that are specific to implicit memory are less robust with the limited number of patients and words possible, and are also less resilient to cultural linguistic heterogeneity.

1 Bradley MM, Lang PJ. *Affective norms for English words (ANEW): Instruction manual and affective ratings*. Technical Report C-1, The Center for Research in Psychophysiology, University of Florida, 1999

2 Coltheart M The MRC psycholinguistic database. The Quarterly Journal of Experimental Psychology Section A 1981; 33: 497-505

The retrieval task should occur 60 minutes after arrival in the CRU. Ideally, the person who read the commands should be the person who performs the retrieval task. Care should be taken to read the word pairs evenly, with no significant tonal or emphasis differences. Subjects should be randomized to be read either Sequence X or Sequence Y.

Instructions: *"Now, we are going to do an exercise where I read you two words. I want you to pay attention to whether one of these words seems to be more familiar to you than the other. If you do have a sense that one seems more familiar to you, tell me that word. If neither word seems more familiar, then please choose one of the words randomly."*

RETRIEVAL LIST X

(1)	**VIOLIN**	**BASKET**
(2)	**EGG**	**FORK**
(3)	**TRUCK**	**HAT**
(4)	**SOUTH**	**NORTH**
(5)	**HISTORY**	**OFFICE**

(6)	**WATER**	**GLASS**
(7)	**TABLE**	**STREET**
(8)	**PENCIL**	**BUTTER**
(9)	**ENGINE**	**JOURNAL**
(10)	**BOWL**	**LAMP**
(11)	**FISH**	**SHEEP**
(12)	**STOVE**	**CLOCK**
(13)	**BIRDS**	**BEARS**
(15)	**INDUSTRY**	**MATERIAL**
(14)	**WOOL**	**SEA**
(16)	**TREES**	**STONES**

RETRIEVAL LIST Y

(1)	**BASKET**	**VIOLIN**
(2)	**FORK**	**EGG**
(3)	**HAT**	**TRUCK**
(4)	**NORTH**	**SOUTH**
(5)	**OFFICE**	**HISTORY**
(6)	**GLASS**	**WATER**
(7)	**STREET**	**TABLE**
(8)	**BUTTER**	**PENCIL**
(9)	**JOURNAL**	**ENGINE**
(10)	**LAMP**	**BOWL**
(11)	**SHEEP**	**FISH**
(12)	**CLOCK**	**STOVE**
(13)	**BEARS**	**BIRDS**
(15)	**MATERIAL**	**INDUSTRY**
(14)	**SEA**	**WOOL**
(16)	**STONES**	**TREES**

Tetanic Stimulus:

In deeply sedated individuals (OAA/S 1), we will apply a tetanic stimulus for 10s at 50Hz 50mAmp to the forearm using a clinical nerve stimulator (EZstim II, model es400, Life Tech). Peripheral nerve stimulation is routinely used on emergence from anesthesia to monitor return of muscle function. It is a moderately painful stimulus and hence we will not conduct it in wakeful individuals. However it has been used to assess rousability during anesthesia previously[9].

Video Recording:

Participants will be video recorded during the duration of each sedation appointment to ensure quality of research and to aid in analysis of the timing events following sedation. A written copy of the recordings will be made for use in the research. Video recordings will be stored in a secure departmental

drive only accessible to study team, and the video will be deleted from the video camera immediately following sedation.

5.1. Allocation to intervention

Each sedative will be administered at different sessions on different days. The first sedative session will be dexmedetomidine followed by ketamine, propofol (dependent on the availability of the drugs) and then midazolam. Allocation is not randomized to ensure that dexmedetomidine, the drug that is easiest to titrate, is administered first. Hence we will recruit 20 patients per drug in a non-randomized design. Total enrollment in the study will be 80 patients. After 20 patients are enrolled into the dexmedetomidine session, no further patients will be enrolled to that drug. Rather we will complete the sessions in order above, ensuring 20 subjects allocated to each.

6. Subject recruitment and consent

6.1. Subject identification

Subjects will be recruited via advertisements (mass email distribution). All advertising materials will be submitted to the IRB for approval. Potential subjects may respond to advertisements via phone to study staff.

6.2. Screening

Subjects will respond to the volunteer posts for the study by calling into a study specific phone number. They will complete a phone screening to determine basic eligibility for the study. If they qualify, the volunteer will be asked to come into UWHC for an in person meeting with a Faculty anesthesiologist and consented, followed by a basic pre-anesthesia exam to confirm they are healthy and safe enough to take part in the study. The Faculty anesthesiologist will confirm with the subject that the study could kill them and there is no direct benefit to the subject.

6.3. Recruitment and consent

The subject will meet with a staff anesthesiologist in a private room. The subject will undergo informed consent discussion for the study where the risks of anesthesia, and the study procedures are explained by an anesthesiologist. Participants will be offered informational handouts on the sedative drugs. Undue coercion will be prevented by stressing that the potential subject does not have to agree to participation and that the future care of the potential subject will not change regardless of the decision about participation.

7. Activities and measurements

Screening & Baseline:

An informed consent discussion, reviewing the risks of participation, eligibility, and data collection will be completed at this visit. All questions from the potential subjects will be answered at this visit. If consent is provided and criteria for inclusion are met, a history and physical, neurologic and anesthesiologic examinations for candidacy will be performed (assessment of a normal airway and adequate venous access). Vital signs will be measured and a review of concomitant medications will be performed.

Sleep Study Protocol:

This protocol mirrors IRB approved protocol (2013-0019; PI Tononi). Subjects will be reimbursed $50 for the nights recording.

Subjects who have never had an EEG or sleep recording may be offered the option of an accommodation night. Subjects will either have an "accommodation" night in the sleep lab, or will be able to use a "demo" hd-EEG net at home prior to the sleep lab recording.

Accomodation night: Subjects will have sleep hd-EEG recordings on two nights. The first night will be an "accommodation" night and the second will be considered the baseline recording. Procedures and recording equipment will be the same between the two nights.

"Demo" hd-EEG nets: Another option to allow accommodation to the EEG net is the use of a "demo" net. The demo nets have the identical appearance and shape of the recording hd-EEG nets (supplied by Electrical Geodesics, Inc.). However, instead of recording electrodes, the net has only the plastic/rubber electrode housing, and also does not include the wires/cables necessary for recording. These do not require conductive gel and can be worn by subjects overnight. Because they do not require any setup or other hardware, the demo nets can be used by a subject at home. Subjects will come to the lab for an instructional session (about 15-20 minutes) to learn how to put on and care for the net. Subjects will then be allowed to take the net home and use it while sleeping for 1-7 nights prior to their scheduled laboratory hd-EEG session. There is no risk associated with the use of or sleeping with this demo net, and has the advantage of improving subject comfort and accommodation to the recording net prior to the laboratory session. Nets that were previously used as recording nets but are no longer functional may also be used as "demo" nets.

EEG is a non-invasive technique commonly used both in research and clinical settings to measure electrical activity in the brain. We will record hd-EEG while subjects are awake and asleep to have recordings for both states. Baseline recordings will be compared to recordings during other experimental procedures. The sleep hd-EEG recordings will be performed in a manner comparable to clinical polysomnography (PSG). The consent will instruct subjects that the PSG should not be considered to be a substitute for a clinical study ordered by their physician. Interpretation of PSG data will be supervised by Dr. Plante, a board-certified Sleep Medicine Specialist. Subjects will be informed of any identified sleep disorders, and assistance will be given with referrals for appropriate medical followup.

In addition to the hd-EEG net, there will be additional electrodes/sensors to monitor other physiologic activity during sleep including:
Electrodes at the corner of each eye to monitor eye movements.
Electrode on the chin to measure muscle activity.
Low-resolution video recording using an infrared camera to allow assessment of sleep/wake state, sleep-related movements, assist with sleep scoring of the EEG record, and to allow real-time monitoring of subjects. In addition to providing important information for assessing sleep, video monitoring provides additional subject safety by allowing visual contact at all times without disrupting the subject.

The actigraph is a small device (about the size of a postage stamp) worn like a watch. The device registers movement, and is used to estimate how many hours a subject sleeps (e.g. – when they are not moving). Actigraphs are FDA approved for use in clinical sleep labs. There is no foreseeable risk associated with wearing this small recording unit. The actigraphic recording will be used to confirm daily (circadian) patterns of activity/sleep, in conjunction with subject report on the Sleep Diary form.

After baseline waking recordings, a sleep EEG will be recorded for the subjects' entire night of sleep. The experiment will be scheduled so subjects lie down and try to fall asleep within 30 minutes of their usual bedtime. Subjects will be in a private room with the door closed and lights off. The subject can contact the investigator present at any time via an intercom. Thereafter the subject will be allowed to sleep undisturbed for 2-3 hours to establish the peak delta power slow wave activity power in natural sleep. After this period, The wake up procedure will be followed with wake ups approximately every 15-30 minutes as per our prior work with the same questions asked as during sedation. After awakening, subjects will repeat the awake eyes open and eyes closed recordings (~10 minutes). The setup and awake recordings will take up to approximately 2-3 hours.

This pilot sub study will establish whether peak slow wave activity during sleep +/- 10% can be used to predict consciousness during sedation and whether EEG characteristics are similar or not between sedation and sleep. Subjects will be reimbursed $50 for the nights recording.

Sedation Protocols:

On a separate day to the baseline data acquisition the subjects will be invited back for the first sedation experiment (dexmedetomidine). Subsequent sedation experiments (for drugs in order), will occur on separate days at least 28 days apart for a given subject (only one study sedative will be administered on a given day). Subjects will be reconsented on the day of sedation. If the patient is inappropriate for one sedative, due to a specific contraindication to that drug (and not the study in general) but are eligible for another drug, then they will progress through the order of sedatives (as above).

Female patients will have a pregnancy test on the day of the sedation to confirm they are not pregnant.

As in our previous protocol (IRB: Pearce (PI) 2008-0018) at the beginning of this session, participants will affirm that in the interval since their baseline visit, they have not acquired any of the cited exclusion criteria that would preclude participation. A review of these exclusion criteria will be completed by the Anesthesiologist. A standard pre-anesthetic assessment will be performed, including confirmation of NPO status, and documented using the standard Department of Anesthesiology pre-operative evaluation form. Participants will have the EEG cap placed and will be given a clear antacid (Bicitra), and a 20-gauge intravenous catheter will be placed for drug delivery. Supplemental oxygen will be provided at 2-10 L/minute via nasal cannula. Many of the sedation drugs have been associated with nausea and vomiting, therefore each subject will be given Ondansetron (8 mg) intravenously as a nausea prophylactic during each procedure as required. Ondansetron has limited side effects that are rarely reported but include headache and constipation. The subject's EKG, non-invasive blood pressure, SaO2, exhaled CO2, and axillary skin temperature will be monitored continuously. Before each experiment, the anesthesia machine and drug cart will undergo routine pre-anesthetic scrutiny, and the availability of resuscitation drugs and equipment will be confirmed and documented.

An anesthesiologist whose sole responsibility is participant welfare will administer the sedatives and will be responsible for monitoring physiological status and documenting vital signs on the standard UW Hospital and Clinics Anesthesia Record. Level of consciousness will be assessed using the 5-point OAA/S scale. The level of 1 corresponds to "no eye opening or coherent response to verbal command or mild physical stimulus (mild shaking or prodding)". At this level of responsiveness, airway patency and ventilation are maintained in most, if not all, patients. Patients will not be routinely intubated for this study.

All anesthetic drugs that are being used in this study are FDA approved clinical anesthetics (propofol, dexmedetomidine and ketamine) in routine clinical use with a proven track record in clinical practice. They will be used in a manner analogous to clinical care with appropriate safety measures, dosing and treatments for side effects available. Contraindications to these drugs are rare but are a critical exclusion criteria. Anesthesia will be administered in a designated clinical anesthesia area in UWHC designed for this purpose: the lithotripsy suite (C7/214) in the Ambulatory Procedure Centre (APC) or the Motility room (C7/201) also in the APC. Both rooms are designated clinical anesthesia areas, with the same access to equipment/emergency care. Drugs will be administered consistent with American Society of Anesthesiology and UW-Madison safety guidelines with full safety monitoring and access to clinical resuscitation equipment. Lactated Ringers or normal saline may also be administered anywhere from 25mL/hr to 1000mL/hr at any point as needed. An anesthesiologist will be present throughout the study procedures until the patient is awake. In the APC, other faculty will be notified of the sedation in case of patient safety issues. If patient safety issues arise they will be managed in the standard clinical manner including an "Anesthesia stat" call if required. Recovery from anesthesia will occur in a designated clinical area for that purpose. All the sedative agents may provoke cardiorespiratory disturbance hence the drugs will be administered slowly, by an anesthesiologist, in clinically approved environments. Full resuscitation equipment will be available throughout.

In order to achieve more controlled transitions in drug concentrations, and therefore more stable levels of sedation when administering intravenous medications (dexmedetomidine, ketamine and propofol), the sedative infusion rate will be altered using information from Target Controlled Infusion (TCI) technology. TCI is a mature technology that is used throughout the world[10-15]. It maintains stable plasma concentrations by adjusting infusion rates according to well established pharmacokinetic models. In addition to providing greater stability[12,13], this method minimizes the need for bolus administration[11] and so reduces unwanted drug accumulation. We propose to use Rugloop TCI software, which is classified as "non-significant-risk". It will be used only as an adjunct to clinical supervision of drug dosing by the anesthesiologist. TCI has been used in multiple volunteer and clinical studies safely[10-15]. **Importantly, we will not deviate from approved FDA total drug doses.** TCI will enable the subject to stay in a stable sedative state allowing cognition to vary while drug concentrations are held constant. We will still be able to use all the prior data collected in the study by retrospectively calculating the drug concentrations using the same pharmacokinetic models.

Dexmedetomidine:

Dexmedetomidine is an ICU sedative noted for its safety and lack of idiosyncratic drug reactions. All adverse effects are dose-dependent and hence dexmedetomidine will be given through slow intravenous infusion[16]. Dexmedetomidine is known for lack of respiratory depression and preservation of airway responses but is known to induce bradycardia and hypotension. Close attention to these parameters will be maintained and treated as per standard anesthesiology measures as required. If

interventions are required, dexmedetomidine infusion will be stopped. Dexmedetomidine has limited effects on seizures, though it has been used safely for resection of seizure foci in patients undergoing awake craniotomy[17], indicating it is safe in patients prone to seizures. Severe allergic reactions to dexmedetomidine have not been reported however, as with all anesthesia care, full resuscitation equipment will be available. Allergic skin reactions to dexmedetomidine have been reported twice both with minimal effects

Dexmedetomidine will be administered by infusion (TCI) (following 15 minutes of acquisition of data in wakefulness as performed previously). The infusion will be conducted consistent with FDA guidance/ approval of up to 1mcg/kg bolus followed by infusion between 0.2-1.4 mcg/kg/hr until the desired OAA/S level is achieved. Incremental changes in infusion rate will be allowed to titrate to the subject's cognitive and physiological state including, for example, slowing bolus administration or bolusing in an incremental fashion. To aid in guiding these incremental changes, infusion rates will be adjusted using information from the TCI model (Dyck model[18]) and clinical judgment. The TCI model will be asked to predict plasma concentrations of dexmedetomidine at 0.5 ng/ml increments allowing 5-minutes to equilibrate between any step change as we used safely in prior studies. Clinical judgment will be used at all times to prioritise subject safety. The anesthesiologist will monitor the subject throughout to ensure they are safe and the drugs are being administered at an appropriate rate as per clinical practice.

Ketamine:

Ketamine is an anesthetic noted for producing dreaming (disconnected consciousness) that produces limited cardiorespiratory effects and has been used safely in prior EEG studies. Ketamine also preserves airway responses[19]. Dr. Sanders has visited Liege, Belgium to witness their use of ketamine for fMRI and HD-EEG experiments. In volunteer studies, ketamine has been associated with nausea and vomiting and hence we will administer ondansetron 8mg intravenously at the end of the ketamine infusion.

Ketamine will be titrated using up to a 4.5mg/kg bolus over 10 minutes followed by 0.1-0.5mg/min intravenous infusion with 5 minutes between increments and titrated to OAA/S level. This infusion rate may be slowed further or stopped for patient safety concerns. Infusion rates will be adjusted using information from the TCI model (Domino model[20]) and clinical judgment. The TCI model will be asked to predict plasma concentrations of ketamine at 0.5mcg/ml increments allowing 5-minutes to equilibrate between any step change as used safely in prior studies. Clinical judgment will be used at all times to prioritise subject safety. Short-lived emergence delirium (confusion on awakening) may occur on emergence from ketamine anesthesia and this will be managed through standard clinical practice including administration of midazolam (up to 4mg IV) if required. However in most instances these episodes are short lived and can be managed through calm reassurance of the subjects. Allergic reactions to ketamine are extremely rare however, as with all anesthesia care, full resuscitation equipment and supportive medications will be available.

Propofol:

Propofol is a standard anesthetic intravenous induction agent used for sedation for minor procedures in ambulatory settings due to its favorable anesthetic profile and wake-up. While it may be safely administered through bolus injections, we again will an infusion to provide a slow titration of sedation and reduce any safety concerns with the drug. Infusion rates will be adjusted using information from the TCI model (Marsh model[21]) and clinical judgment. The TCI model will be asked to predict plasma concentrations of propofol at 0.5mcg/ml increments allowing 5-minutes to

equilibrate between any step change as we used safely in prior studies. Clinical judgment will be used at all times to prioritise subject safety. Propofol can provoke cardiorespiratory depression and loss of airway reflexes if given too rapidly. We will mitigate these risks through careful drug titration from an anesthesiologist. Subjects will be notified that propofol can initially cause some pain on injection (through their IV line) and if this is not tolerable, the subject can withdraw from the study. Allergic reactions to propofol are rare. There have been concerns that food allergies may cross react with propofol but these have proven unfounded[22]. Nonetheless, while a reaction is considered unlikely, clinical grade resuscitation equipment will be available.

Propofol dosing will be confined to FDA approved limits with up to a 2.5mg/kg bolus over 1 to 3 minute followed by an infusion of 50-200mcg/kg/min titrated to the desired OAA/S levels.

Midazolam

Midazolam is a sedative/anesthetic benzodiazepine used both for anesthetic maintenance and sedation in critically ill and non-critically ill patients. It is safe to administer through bolus injections or an infusion. We will administer midazolam in accordance with FDA guidance: initial boluses of up to 0.35mg/kg followed by doses up to 0.1mg/kg/h as a maintenance infusion. The side effects of midazolam are similar to propofol, though pain on injection are not noted. Allergic reactions are rare but standard clinical practice measures will be employed to ensure subject safety.

In this session the subject will experience both HD-EEG and auditory stimuli (the Roving Oddball or LGD paradigm) as described above during administration of each sedative. The drugs will be titrated initially to an OAA/S Score of 2-3. During this phase the subject will be asked to report whether he sees different perceptions on visual illusions. We hypothesize that as the subject drifts to sleep, they will lose feedback processing in the brain and this will lead to altered predictive coding.

Observation	OAA/S Score
5	Wake/responds to verbal commands
4	Lethargic/responds to name
3	Responds only after name is called loudly and/or repeatedly
2	Responds only after mild prodding or shaking
1	Does not respond to mild prodding or shaking

Once at a stable sedative OAA/S level 2-3 for 5 minutes we will rouse the patient to wakefulness though verbal command or mild shaking, and ask the patient to report whether they were awake prior to us contacting them, whether they heard the tones through headphones they were wearing and to respond to the questions below:

1. What was going through your mind before awakening?
2. Are you sure? Anything else to report? Did you see faces?
3. Did time pass?
4. Did you hear the tones/words? Were you aware of your environment?
5. Were you asleep or awake? (Visual Analogue Score 0-6)

This will provide information about whether they were conscious and what they were conscious of. Specifically we will record whether they were conscious of their environment (connected consciousness), dreaming (disconnected consciousness), conscious but cannot remember details, unconscious (no report). We will also ask whether they noticed time passing. We anticipate the questions will take 15s and in the first 4-6 arousals the predictive coding experiments will be conducted. The patient will then have to return to OAA/S level 2/3 for at least 45 seconds before repeating the questions. This will be continued with arousals at a maximum of every minute for 60 minutes however we anticipate that the patients will be questioned less frequently as it will take time to fall asleep again. Total recording from the auditory task will be 60 minutes. An additional 60 minute period of EEG recordings will be undertaken immediately after. For subject comfort we will interleave these periods.

The subjects will then be more deeply sedated to OAA/S 1 and 20 minutes of auditory stimulation and 20 minutes of EEG recorded. In this section we will also conduct 20 minutes of recording of response to tetanic stimulus using a 10 second, 50Hz, 50mAmp forearm stimulus. 5 seconds before and after the tetanic stimulus the subject will be asked to squeeze the observer's hand and open their eyes and then asked to squeeze again if they have pain mimicking our recent isolated forearm technique study demonstrating that 4.6% of subject become wakeful after intubation under general anesthesia. If they become awake enough to answer the questions above, these will be repeated. A maximum of 20 stimuli will be administered over 20 minutes (one per minute). The drugs will then be stopped and the EEG continued until the subject is OAA/S 5. At that point we will conduct the Confusion Assessment Method (CAM) to assess for delirium on awakening and monitor the patient until there is absence of delirium on the CAM test. We estimate the whole study duration will be five hours, four of which will be sedation.

Participants will be observed in the clinical environment until they are judged by the anesthesiologist to be ready to move to the UW Clinical Research Unit (CRU) to complete the recovery process. We anticipate the full recovery from the sedatives to take at least two hours. Orders for post-sedation care in the CRU will follow standard post-sedation hospital guidelines for patients after surgery. UWHC clinical criteria for 'street readiness' will be used to determine suitability for discharge after a procedure performed under sedation. Participants will be discharged by the faculty anesthesiologist directly or by delegation to the CRU nurse according to pre-determined criteria. Participants will be given a copy of instructions and contact information in the event that complications arise after leaving the hospital, and they will be mandated to be accompanied home by a companion. Patients will be instructed not to drive or operate machinery, and not to consume other drugs with central nervous system activity within 24 hours of drug-induced sedation as per standard instructions after procedural sedation.

All participants will be contacted by telephone the day following sedation to confirm full recovery and inquire about untoward incidents (e.g. nausea, headache, mood disturbance). This information will be documented on a post-procedure adverse event form.

If patients have been enrolled in the study previously and re-present for another sedation episode they will not undergo re-screening but will have a new consent completed with their prior ID – X, where X is a number that refers to the number of their sedation visits. E.g. if this is the second visit study ID-2.

Sedation Appointment Follow-Up

Participants will be called by study staff the day following their sedation appointment. During this phone call, the participant will be asked about their recovery from sedation and any adverse events will be recorded. During this phone call, the participant will also be asked if they can write a summary of what

they remember from the sedation experiment. They will be asked to email in this reflection, and their response will be stored in a secure research drive within their participant folder.

7.1. Data to be recorded

Demographics (age, sex, right handed or left handed, ASA grade, level of anxiety prior to commencing drug (on visual analogue scale), physiological variables, blood samples for intravenous sedatives plasma concentration (ketamine, propofol, midazolam and dexmedetomidine), Intra-procedural data (responsiveness to questions during sedative procedures, responses to predictive coding tasks, OAA/S status, etc.) Roving Oddball or LGD paradigm details and EEG data will be collected from each subject, recorded in the CRF (see appendix 1), and stored in an anonymized fashion on secure servers.

7.2. Blood sample collection

Patients sedated with ketamine, propofol, midazolam and dexmedetomidine will have two intravenous lines placed for administration of sedatives and rescue medications/blood draws respectively. All of the lines will be placed in preparation on the day of the sedative procedure.

Blood samples for plasma drug levels will be drawn a drug induced OAA/S 2-3 and OAA/S 1 levels are reached. Two 5ml blood samples will be drawn for consistency. Each blood sample will be a 5 mL sample from the intravenous line indicated for blood sampling in EDTA tubes. The total amount of blood drawn for this study will be approximately 80 mL (2*2*5 mL samples for each sedative drug x 4 sedative drugs). The samples will be stored on ice until processed in Dr. Kirk Hogan's Lab. Samples will be spun down to plasma and alliquotted into separate vials labeled with coded subject data and frozen at -80° F until analysis. Further analyses may require processing of de-identified and anonymized data in other laboratories but patient confidentiality will be maintained.

7.3. Data entry

Data collected for this study will be recorded on electronic Case Report Forms (CRFs) (see appendix 1) and stored on secure servers in the Department of Anesthesology. Adverse events will also be recorded in OnCore to facilitate the DMC.

Clinical Trials Management Software:
Online Collaborative Research Environment-Clinical Research Management (OnCore) will serve as the Clinical Trial Management System for this study. ICTR OnCore is supported by the UW School of Medicine and Public Health Information Technology (SMPH-IT) staff and managed by the UW ICTR Clinical Research Infrastructure System (CRIS).

OnCore is a sophisticated, web-based data management system that: a) ensures secure, easy data entry at multiple sites; b) integrates multiple data sources; c) provides controlled, secure access to sensitive data using role-based access control; d) provides workflow automation; and e) allows export and reporting of data for Data and Safety Monitoring Boards and biostatisticians. This software provides protocol management functions (e.g. subject scheduling; screening, data organization), maintains updated forms, addresses budget development, billing, and fiscal management, generates summary reports, and provides essential links with other research administration and electronic medical records systems. ICTR OnCore eases the burden of the individual researcher and unifies protocol management within research programs and across research sites, enhancing protocol integrity and regulatory compliance efforts.

7.4. Subject withdrawals

Subjects that do not meet the EEG signaling criteria (due to craniofacial muscle artifact) will be withdrawn from the study by the Investigator after the baseline visit. Their consent and data collected up to this point will be maintained as part of the study records. Additionally, subjects will be withdrawn if safety concerns arise (see adverse events below).

If a subject is withdrawn from the study prior to any sedative procedures, they will not count towards the enrollment total of 80 subjects, and we will replace them with a complete subject. If a subject is withdrawn for any other reason after at least one of the sedative procedures has been completed, they will remain part of the study total enrollment numbers.

7.5. Stopping rules

In eventualities (such as cardiorespiratory safety effects) the study drug will be stopped immediately. If any degree of ventilatory insufficiency is detected, the anesthesiologist in attendance will adjust the position of the airway and assist ventilation with bag, mask and supplemental oxygen as necessary.

Pulmonary aspiration of gastric contents: Participants who have received an oral clear antacid after an 8-hour fast are at very low risk for pulmonary aspiration. Participants will be closely monitored for signs of nausea and vomiting, and the experiment will be terminated in the event of emesis.

In the event of an allergic reaction, the study drug will be stopped immediately. The subjects will be given chlorpheniramine 10mg, hydrocortisone 100mg and will be reviewed for necessary anaphylaxis protocols. Allergic reactions/anaphylaxis to anesthetic drugs are extremely rare.

If heart rate falls below 40bpm or mean arterial blood pressure falls <55 mmHg or >133 mmHg, the experiment will be stopped and the patient will be treated until stable.

Sedation will be limited to a 4 hour maximum duration, once 4 hours has elapsed the sedation will be stopped and the patient will be woken up.

The study will be stopped as advised by the DMC based on reporting of AQI adverse events to the DMC. Seizures and intubations will be notified to the ICTR DMC within 7 days of the event. The DMC will be informed of study progress on a monthly basis and any serious adverse events including drug reactions will be reported to the DMC within 24 hours of occurrence.

8. Data analysis and statistical considerations

8.1. Sample size determination

Spontaneous EEG: Our primary outcome is difference in delta power (also known as slow wave activity) over posterior cortex between consciousness and unconsciousness during sedation. Our preliminary data suggest that a mean difference in 12 dB with a standard deviation of 12 dB requires 16 subjects per group. An additional 4 subjects per group are recruited may be to difficult to wake patients and get descriptions of the subjects conscious experience through questioning hence approval is sought for the

enrollment of 20 participants per drug in the present proposal. In total 80 subjects will be recruited.

Secondary endpoints: All secondary endpoints are considered exploratory:

1. The incidence of disconnected conscious experience (dreaming) versus connected conscious experience (awareness of the external world) during sedation.
2. The ability to percieve visual illusions (defined as time to perceive the illusion and percentage accuracy of perceiving the illusion) or match auditory and visual cues (defined as the time to match the cues and percentage accuracy of matching the cues).
3. Differences in auditory and visual evoked responses under sedation compared to wakefulness and other conscious states.

Exploratory endpoints:

1. Difference in HD-EEG responses assessed by calculation of the Phase Locking Factor following auditory stimulation between conscious experience under sedation and lack of conscious experience. Phase locking factor is calculated by taking the Hilbert transform of the evoked response and quantifying the alignment of phases based on the imaginery component of the signal. PLF is quantified on a scale of 0 to 1. With 0 being no phase locking and 1 being maximal phase locking. We expect than under anesthesia PLF will decrease across the whole scalp. In our experience evoked potentials have a high signal-to-noise ratio, and statistically valid responses at single sites may be obtained in a single subject by averaging less than 150 single responses. In an earlier TMS investigation of hd-EEG changes during sleep complete response sets from only 6 subjects were required to detect statistically significant differences in hd-EEG results. In our study we will contrast consciousness under sedation (confirmed by report of participant) with lack of report (unconsciousness). The percent difference in duration of Phase Locking Factor (PLF) following auditory stimuli in the consciousness state (PLFc) of anesthesia compared to the unconsciousness state (PLFu) of anesthesia (100*[PLFu-PLFc]/PLFc). Duration of PLF from the conscious and unconscious states will be a single value which has been averaged over all measurements from all channels across the scalp. The primary hypothesis is that at least one of the drugs will show a significant difference in percent difference in duration of PLF between the two consciousness states.

2. Drug effect on percent difference in duration of PLF between the two consciousness states.
3. The effect of consciousness during sedation vs. unconsciousness during sedation on PLF following auditory stimulation.
4. Differences in connectivity between conscious states.

8.2 Sample size and Statistical Analysis Plan

Our primary endpoint as defined above is a single value per subject within drug that takes into account the two different states of consciousness during anesthesia. Splitting our alpha evenly between all 4 drugs leaves us with an alpha level of 0.0125 for each test. If we recruit 16 patients who complete each drug session, we will have 80% power at an alpha level of 0.0125 to identify a 12 dB difference in the delta power between consciousness to unconscious during anesthesia with an expected standard deviation of 12dB using a single sample t-test against a null hypothesis of 0 dB difference. These estimates are made based on pre-acquired data comparing consciousness in ketamine sedation with

deep anesthesia (propofol). We anticipate that in 25% of subjects it may be to difficult to wake patients and get descriptions of the subjects conscious experience through questioning hence approval is sought for the enrollment of 20 participants per drug in the present proposal. In total 80 subjects will be recruited.

A similar power analysis for TMS, which is likely to be similar to auditory stimuli, suggests that we will have 86% power to show a 20% difference in the Phase Locking Factor using a single sample t-test against a null hypothesis of 0% difference. These estimates are made based on pre-acquired data comparing consciousness in wakefulness with deep anesthesia (propofol).

Sedation will be recorded as the Observer Assessment of Alertness/Sedation (OAA/S) scale. Standard clinical monitoring of physiological variables (heart rate, blood pressure, oxygen saturation, respiratory rate, and oxygen saturation) will be recorded. The imaging and EEG endpoints will be analyzed by standard analytical techniques such as Statistical Parametric Mapping to analyze different evoked and induced power between conscious report and lack of conscious report. Following FIR filtering we will calculate the spontaneous delta power at CPz (as well as in the precuneus in source space) as well as PLF per channel as calculated previously[23] and then average all channels across the scalp to get a single value per subject. The difference in delta power (primary endpoint) and the percent difference in duration of PLF following the auditory stimuli between consciousness and unconsciousness will be compared against a null hypothesis of 0dB or 0% difference respectively with single sample t-tests for each drug individually with an alpha level of 0.0125. An exploratory analysis will look for a drug effect in the percent difference in duration of PLF, or other evoked response changes, between the two consciousness states with a repeated measures ANOVA with drug as a fixed effect and subject as a random effect. If we report this analysis we will explicitly state that the subjects were not randomized to each sedative. Other exploratory analysis will look at possible differences in percent difference in duration of PLF following auditory stimulation. This will be analyzed in the same methods as the primary endpoint. Secondary and exploratory analyzes will be conducted at a significance level of 0.05. We acknowledge that we are likely under powered to detect statistically significant differences in our secondary analyses however the results can be foundation blocks for further studies on the differences between consciousness and unconsciousness states of anesthesia. Underpowered exploratory analyses may not be completed if the analysis would not yield meaningful results for future studies.

5. Risks and benefits of trial participation

Overall, risks in this study should be infrequent. Specific risks for individual study procedures are detailed below.

None of the procedures included in this study are considered to be therapeutic or diagnostic, as detailed for each specific procedure. Each of the procedures has minimal risks associated with them, and have been employed successfully, without incident, by our research group for the last 10 years in sleep [Protocols H-2001-456, H-2002-414, H-2003-238, H-2004-0023, H-2004-444, H-2007-0150]. Furthermore we have specifically applied HD-EEG safely during anesthesia (IRB: Pearce (PI) 2008-0018) and seek to extend these findings here.

a. Potential risks

DRUGS

Bicitra

Bicitra may cause diarrhea, nausea and vomiting, hypernatremia and convulsions. Bicitra may also decre serum calcium levels.

Ondansetron
Ondansetron can cause constipation, diarrhea, and headache.

Propofol
Propofol can provoke cardiorespiratory depression and loss of airway reflexes resulting in airway obstruction if given too rapidly. Propofol may also cause hypotension, hypertension, apnea, oxygen desaturation, arrhythmia, stinging at injection site, rash and pruritus. Allergy to propofol has been reported but is rare.

Ketamine
Short-lived emergence delirium (confusion on awakening) may occur on emergence from ketamine anesthesia and this will be managed through standard clinical practice including administration of midazolam (up to 4mg IV) if required. Other common side effects may include hypotension, bradycardia, arrhythmia, apnea, laryngospasms, diplopia, nystagmus, increased intraocular pressure, and aspiration.

Dexmedetomidine
Dexmedetomidine is known for lack of respiratory depression and preservation of airway responses but is known to induce bradycardia, dry mouth and hypotension. Close attention to these parameters will be maintained and treated as per standard anesthesiology measures as required. If interventions are required, dexmedetomidine infusion will be stopped.

Midazolam
Midazolam may cause cardiorespiratory depression and loss of airway reflexes resulting in airway obstruction if given too rapidly. Other common side effects include hiccoughs, headache, nausea, vomiting, coughing and drowsiness.

DRUGS TO COUNTERACT COMMON SIDE EFFECTS:
Bradycardia:
Glycopyrrolate
Atropine

Tachycardia:
Labetralol
Metoprolol

Hypotension:
Ephedrine
Phenylephrine

Hypertension:
Labetralol
Metroprolol
Hydrazine

EEG:
Electroencephalography is a technique commonly used to non-invasively measure electric brain activity both in research and in clinic. There is no risk from EEG recording. However, individuals with very sensitive skin may experience, on rare occasion, a slight irritation at the site of sensor application due to the use of mildly conductive saline gel and site preparation for EEG recording. Continuous EEG monitoring during auditory stimulation represents an additional safety factor allowing the early detection of any sign of EEG abnormality. The hd-EEG recording device will be screened for current leak before use in the operating room.

Intravenous Catheters:
Intravenous catheters will be placed by the attending anesthesiologist using local anesthesia before needle placement. Nevertheless there may be some discomfort to participants upon insertion, and there may be a slight risk of bruising, and inflammation after removal. In normal subjects subcutaneous infiltration and induration are very rare complications, and are easily managed with warm packs and oral analgesics.

Respiratory depression / loss of patent airway:
Because the anesthetics will be administered incrementally under close observation, we expect that if respiratory depression occurs it will be limited in depth and duration. In general, a reposition of the airway (i.e., jaw thrust) and assisted ventilation with bag and mask will be sufficient to return the participant to full ventilatory control within several minutes. If greater assistance is required, a standard oral, nasal and laryngeal mask airways will be immediately available. Caregivers are skilled in their use. Full resuscitation equipment will also be available if required.

Pulmonary aspiration of gastric contents:
Participants who have received an oral clear antacid after an 8-hour fast are at very low risk for pulmonary aspiration. Participants will be closely monitored for signs of nausea and vomiting, and the experiment will be terminated in the event of emesis. Participants will be sedated in the operating room on tables allowing positions that reduce the risk of aspiration even further, and a fully operational suction apparatus will be available at all times. During recovery, participants will be closely observed for signs of aspiration, and instructed in the steps to take if signs of aspiration appear after discharge.

Cardiovascular Effects:
All the sedative drugs can provoke cardiovascular changes including hypotension and hypertension, bradycardia and tachycardia and arrhythmias. These may be most pronounced for dexmedetomidine. If these arise they will be treated in line with standard clinical care including administration of medications and fluids as clinically appropriate.

Prolonged sedation:
It is anticipated that most, if not all, participants will have fully recovered within 5 hours after receiving the sedative drugs. Patients will be instructed not to drive or operate machinery, and not to consume other drugs with central nervous system activity within 24 hours of drug-induced sedation as per standard instructions after procedural sedation.

Drug allergy and other side effects:
Allergies to ondansetron, propofol, ketamine, midazolam and dexmedetomidine administration are extremely rare. Patients with known allergy will be excluded. While a number of side effects have been report with acute and chronic use, they are very uncommon. Common or serious risks include residual

'hangover' after administration of midazolam, such as sleepiness, and impaired psychomotor and cognitive functions that may persist into the next day, and may impair the ability of users to drive safely.

Risks associated with loss of confidentiality
There is a risk that information recorded about subjects will be shared with people who would not normally have access to this information.

Unknown risks
This study may involve risks to the subject which are currently unforeseeable. We will inform subjects as soon as possible if we discover any information that may affect the subject's health, welfare, or decision to be in this study.

b. Mitigation of potential risks

An anesthesiologist will ensure that subjects fit the inclusion criteria for the study and should not be excluded for safety reasons. The Anesthesiologist will be present throughout the study to ensure safety at clinical standards. If it is judged the patient is at risk from continuation of the study, the study will be stopped. Patients who are at risk from seizure induction will be excluded.

Mitigation of risks associated with EEG: There are no known risks from EEG.

Mitigation of risks associated with Sedation: The Anesthesiologist will be present throughout the study to ensure safety at clinical standards including administration of required medications and fluids to maintain subject safety. Clinical grade anesthesia and resuscitation equipment will be available throughout the study. Subjects will be instructed not to drive or operate heavy machinery and must be accompanied by someone who can take them home or schedule a ride from another service. The study coordinator will verbally confirm this with the subject. The study coordinator will also instruct the subject to be accompanied overnight and verbally confirm this will happen with the subject.

Mitigation of risks associated with Blood Draws: Staff obtaining blood samples will have the necessary training and draw samples from existing venous catheters.

c. Potential benefits and risk-to-benefit ratio

No direct benefit will accrue to participants. We anticipate that society may benefit from the possible development of a new diagnostic technique (i.e., a monitor of sedation and anesthesia), and because information may be obtained regarding the mechanisms of conscious experience that has important ramifications for detecting/preventing consciousness during anesthesia and sedation and in neurological conditions such as unresponsive wakefulness syndrome and coma. Furthermore in our recent ConsCIOUS study, 4% of patients during routine general anesthesia were aware of intubation (connected consciousness). Half of these, 2% of all people were in pain. This is a good example of why it is important for us to understand the mechanisms and markers for conscious experience that is related to the external world (connected consciousness). In order to identify the markers and mechanisms of connected consciousness, it is important to perform "within state" experiments such as probing conscious experience during sedation, as we plan here. If we are able to identify a marker of sensory disconnection or unconsciousness in these studies this will have great potential importance for the clinical care of patients under anesthesia and sedation. These potential benefits go beyond anesthesiology and may inform diverse fields from the disorders of consciousness (such as coma or delirium) to sleep. Finally once we identify a potential marker, studies can be undertaken to understand the mechanisms of sensory connectedness under anesthesia/sedation followed by studies to prevent sensory connectedness under anesthesia/sedation through targeting these mechanisms. It is also important to separate connected consciousness (experience of external world) from disconnected consciousness (dreams). If an anesthesia monitor could detect disconnected consciousness (e.g. dreaming as occurs in 10-30% of people during emergence from anesthesia) then this might imply to the care provider that they should deepen the anesthetic but this would not be necessary as the patient is unaware of the surgery. Hence a marker of sensory disconnection/connectedness (to provide an index of likelihood of awareness of surgery) will provide important information to guide the clinical care of patients undergoing anesthesia and sedation. Overall our work aims to identify markers of conscious experience under anesthesia, whether it is connected or disconnected, and use these markers to define clinical monitoring devices and novel pharmacological strategies to reduce patient experience of surgery under anesthesia.

6. Adverse events and unanticipated problems

a. Adverse event definitions

Adverse event (AE)

An adverse event is defined as any untoward or unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research. Patients will be phoned 24 hours after discharge to confirm a lack of adverse events.

Serious adverse event (SAE)

A serious adverse event is defined as any adverse event that meets one of the following criteria:

- Results in death; OR
- Is life-threatening; OR
- Requires hospitalization or prolongs existing hospitalization; OR
- Results in significant or persistent disability or incapacity; OR
- Results in a congenital anomaly/birth defect; OR

Unanticipated problem (UP)

An unanticipated problem is defined as an event that meets all of the following criteria:

1) Unexpected in severity, nature, or frequency given the research procedures and the characteristics of the subject population (i.e., problems that are not described in this protocol or other study documents); AND
2) Related or possible related to participation in the research; AND
3) Suggests that research places subjects or others at a greater risk of harm related to the research than was previously known or recognized.

b. Reporting period

Any adverse events or serious adverse events will be reported within the timeframes outlined by UW-Madison.

c. Severity assessment

The severity of all adverse events will be assessed according to the following scale:

- Mild = not requiring treatment
- Moderate = resolved with treatment
- Severe = inability to carry on normal activities and required professional medical attention

d. Causality assessment

The Site PI will determine the relationship of adverse events to the research intervention using the following scale:

- Definite = AE is clearly related to the study procedures
- Probable = AE is likely related to the study procedures
- Possible = AE is possibly related to the study procedures
- Unlikely = AE is doubtfully related to the study procedures
- Unrelated = AE is clearly not related to the study procedures

e. Procedures for recording and reporting adverse events

Adverse events will be recorded in the medical record and reported to the IRB. AEs and UPs will also be entered into OnCore for review by the DMC.

f. Other reportable events

Reporting timeframes begin when the site learns of the occurrence of the event.

Event	Definition	Reporting
Breach of confidentiality	The exposure of any study information or communications directly related to a study subject to anyone not named as study staff or the release of a study subject's identifiable information to study staff who were not specified to receive such information in the protocol or IRB application.	Treat as major deviation

Event	Definition	Reporting
Protocol deviation	A deviation is an incident involving a departure from the IRB-approved protocol in the actual conduct of the study. Deviations may result from the action of the participant, investigator, or staff.	See below
Major deviations	Deviations are considered major when the unapproved change(s) in previously approved research activities, implemented without IRB approval, may potentially adversely affect subjects' rights, safety, welfare, or willingness to continue participation, or affect the scientific design of the study and/or the integrity of the resultant data.	Treat as an Unanticipated Problem
Minor deviations	Deviations are considered minor when the unapproved change(s) in previously approved research activities, implemented without IRB approval, do not adversely affect subjects or the integrity of the study data.	Sites are to report cumulative events to AE Coordinator at time of continuing review.
Protocol violation	An incident involving an intentional deviation from the IRB-approved protocol that was not implemented in response to an emergency situation and that may impact a subject's rights, safety, and/or welfare, makes a substantial alteration to risks to subjects, or affects the scientific design of the study and/or the integrity of the resultant data. Violations may also be repeated deviations (major or minor) of the same nature. Violations can represent serious or continuing non-compliance with the federal regulations and guidelines for ethical conduct of human subject research.	Treat as an Unanticipated Problem
Protocol Exceptions	A protocol exception is an IRB-approved deviation for a single subject or a small group of subjects, but is not a permanent revision to the research protocol.	Protocol exceptions must be approved by local IRB prior to implementation.

g. Reporting by study site to local IRB

The study site will follow their local IRBs guidance for reporting all events to the local IRB.

7. Data Safety Monitoring

We plan to utilize the UW Institute for Clinical and Translational Research (ICTR) Data Monitoring Committee (DMC) to oversee the study. The UW ICTR DMC is comprised of experienced members (core plus ad hoc) with expertise required to oversee this study. The DMC members will review protocol-specific reports created by statisticians using data pulled from the ICTR OnCore clinical research

management system. These standard reports will include an overview of study objectives, a review of actual and projected accrual rates, an evaluation of patient demographics for balance of randomization, and a summary of the number and seriousness of adverse events. An interim analysis of study results may be performed and source documents may be reviewed to allow the DMC to independently judge whether the overall integrity and conduct of the protocol remain acceptable based on data provided and reported by the Principal Investigator. The DMC will make recommendations to the Principal Investigator that could include actions of continuation, modification, suspension, or termination.

In providing oversight for the conduct of this study, the ICTR DMC will meet biannually. Additional meetings may be scheduled as determined by the DMC or as requested by the PI.

8. Medical Monitor

The Medical Monitor may perform oversight functions (e.g., observe recruitment, enrollment procedures, and the consent process for individuals, groups or units; oversee study interventions and interactions; review monitoring plans and reports; and oversee data matching, data collection, and analysis) and report their observations and findings to the sponsor or a designated official.

The Medical Monitor may discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research. The Medical Monitor shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects. Medical Monitor shall have the responsibility to promptly report their observations and findings to the sponsor or other designated official.

Each participating site's local IRB must approve a written summary of the Monitors' duties, authorities, and responsibilities.

The Medical Monitor shall have expertise consonant with the nature of risk(s) identified within the research protocol, and shall be independent of the team conducting the research involving human subjects.

The Medical Monitor is required to review reports of serious adverse events (SAEs) and unanticipated problems (UPs) and provide an independent evaluation and an unbiased, written report of each event. At a minimum, the Medical Monitor shall comment on the outcomes of the event or problem, and in the case of an SAE comment on the relationship to participation in the study. The Medical Monitor shall also indicate whether he or she concurs with the details of the report provided by the site investigator. The Medical Monitor's evaluation of these events will be provided to each participating site's local IRB.

If requested, the Medical Monitor shall have access to de-identified source documents.

The Medical Monitor for this trial is Dr. Corey Amlong.

9. Administrative requirements

a. Good clinical practice

The study will be conducted in accordance with FDA and ICH guidelines for Good Clinical Practice. All study staff will be thoroughly familiar with the contents of this protocol and associated trial materials.

b. Data quality assurance

Our study team will prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each study subject. Study data will be entered into an electronic case report form (eCRF) by site personnel.

c. Ethical consideration

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the subjects. The study will only be conducted at sites where IRB approval has been obtained. The protocol, informed consent form, written information given to the patients, safety updates, annual progress reports and any revisions to these documents will be provided to the IRB by the investigator.

d. Patient confidentiality

All subjects will be assigned a study-specific ID number. We will maintain a master list linking each subject's medical record number (MRN) with a study-specific ID number. This list will be maintained in a location separate from any study data. Only site staff listed on the IRB application shall have access to the list.

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

e. Investigator compliance

The investigator will conduct the trial in compliance with the protocol approved by the IRB. Changes to the protocol will require written IRB approval prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to subjects.

f. Subject cost and payment

Cost

Subjects will not incur any costs associated with their involvement in this study.

Payment

Subjects will be paid $50 at their baseline visit, $50 for the sleep study, and $200 at each of the sedative procedure visits.

10. Funding sources

The funding for this protocol will be provided by the Investigator's start-up funds.

11. Publication Policy

This data from this study may be used for presentation at local, regional, national or international meetings or for publication in a peer-reviewed journal.

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Appendix 1: UNCONSCIOUS CRF

UNCONSCIOUS CRF

Date: **Age:**

Initials:

Study ID: **ASA:**

Circle: **Right Handed** **Left Handed**

(BASELINE) **TIME** **EEG TIME**

COMMENTS:

Start: 0:00:00

_____ EEG	TIME	EEG TIME	EYES
Start:			
End:			
_____ EEG			
Start:			
End:			
_____ EEG			
Start:			
End:			
_____ EEG			
Start:			
End:			
_____ EEG			
Start:			
End:			
_____ EEG			
Start:			
End:			

(PREDICTIVE CODING)

TP:____________
Start:
End:
Break time: (

TIME | **EEG TIME**

TP:____________
Start:
End:
Break time: (

TIME | **EEG TIME**

TP:____________
Start:
End:
Break time: (

TIME | **EEG TIME**

TP:____________
Start:
End:
Break time: (

TIME | **EEG TIME**

TP:____________
Start:
End:
Break time: (

TIME | **EEG TIME**

TP:____________
Start:
End:
Break time: (

TIME | **EEG TIME**

Strategy:

Observations/Notes:

(PREDICTIVE CODING)

TP:___________ **TIME** **EEG TIME**

Start:
End:
Break time: (

TP:___________ **TIME** **EEG TIME**

Start:
End:
Break time: (

TP:___________ **TIME** **EEG TIME**

Start:
End:
Break time: (

TP:___________ **TIME** **EEG TIME**

Start:
End:
Break time: (

TP:___________ **TIME** **EEG TIME**

Start:
End:
Break time: (

TP:___________ **TIME** **EEG TIME**

Start:
End:
Break time: (

Observations/notes:

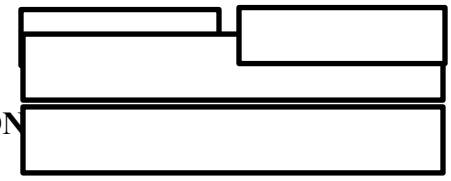
(SEDATION START)

Level of anxiety prior to drug administration:
1 2 3 4 5 6 7 8 9 10

TIME **EEG TIME**

Start:
BOLUS

INFUSION



Observations/notes:

(WAKE REPORT)

	TIME	EEG TIME
Start:		
DOSE:		

(RESTING EEG)	**TIME**	**EEG TIME**
Start:		
End:		

(ODDBALL)	**TIME**	**EEG TIME**
Start:		
End:		

(LGD)	**TIME**	**EEG TIME**
Start:		
End:		

WAKE # ____

	TIME	**EEG TIME**
Time Approached (voice):		
Time Approached (touch):		
Time nerve stim:		

(Duration of nerve stim_____seconds)

Time Response:		

Observations/notes:

WAKE #
OAA/S SCORE ☐

What was the last thing going through your mind before I spoke to you? + Were you having a dream or were you awake? – Do you think you were unconscious?

Are you sure? Anything else to report? Any faces? Moving?

Did time pass since the last time we spoke? +How much time has passed since we last spoke?

Did you hear the tones/words? Were you aware of the external world?

Were you asleep or awake? Yes/no +Visual Analogue Score 0-6

(END SEDATION)

	TIME	EEG TIME
End:		

Did you feel confused as you woke up?

(Wait 10s after nerve stim) **Go to Command Sequence: _____**
Number of times repeated first command until response: _____

COMMAND List A.:

"[NAME], **BIRDS fly NORTH**."
Followed by "[NAME], **squeeze my hand**."
Followed by " **TABLE"**
Followed by "[NAME], **if you are in pain squeeze my hand two times**."
Followed by " **HAT**"
Followed by "[NAME], **if you are ok squeeze my hand two times**."
Followed by " **PENCIL**"
Followed by "[NAME], **show me two fingers**"
Followed by " **LAMP**"
Followed by "[NAME], **if FISH swim in the SEA, squeeze my hand"**
Followed by " **STOVE**"
Followed by "[NAME], **if STONES float on WATER, squeeze my hand**."
Followed by " **BASKET**"
Followed by "[NAME], **squeeze my hand**."
Followed by " **INDUSTRY**"
Followed by " **OFFICE**"
Followed by " **EGG**"
Followed by " **JOURNAL**"
Followed by "[NAME], **squeeze my hand**."

If the patient keeps responding, please repeat the sequence of commands and inform the care provider.

COMMAND List B:

"[NAME], **squeeze my hand**."
Followed by "[NAME], **BIRDS fly NORTH**."
Followed by " **TABLE"**
Followed by "[NAME], **if you are in pain squeeze my hand two times**."
Followed by " **HAT**"
Followed by "[NAME], **if you are ok squeeze my hand two times**."
Followed by " **PENCIL**"
Followed by "[NAME], **show me two fingers**"
Followed by " **LAMP**"
Followed by "[NAME], **if FISH swim in the SEA, squeeze my hand"**
Followed by " **STOVE**"
Followed by "[NAME], **if STONES float on WATER, squeeze my hand**."
Followed by " **BASKET**"
Followed by "[NAME], **squeeze my hand**."
Followed by " **INDUSTRY**"
Followed by " **OFFICE**"
Followed by " **EGG**"
Followed by " **JOURNAL**"
Followed by "[NAME], **squeeze my hand**."

COMMAND List C:

Followed by "[NAME], **BEARS walk SOUTH**."
Followed by "[NAME], **squeeze my hand**."

Followed by “ **STREET”**
Followed by “[NAME], **if you are in pain squeeze my hand two times**.”
Followed by “ **TRUCK**”
Followed by “[NAME], **if you are ok squeeze my hand two times**.”
Followed by “ **BUTTER**”
Followed by “[NAME], **show me two fingers**”
Followed by “ **BOWL**”
Followed by “[NAME], **if SHEEP have WOOL, squeeze my hand”**
Followed by “ **CLOCK**”
Followed by “[NAME], **if TREES are made of GLASS, squeeze my hand**.”
Followed by “ **VIOLIN**”
Followed by “[NAME], **squeeze my hand**.”
Followed by “ **MATERIAL**”
Followed by “ **HISTORY**”
Followed by “ **FORK**”
Followed by “ **ENGINE**”
Followed by “[NAME], **squeeze my hand.”**

If the patient keeps responding, please repeat the sequence of commands and inform the care provider.

COMMAND List D:

Followed by “[NAME], **squeeze my hand**.”
Followed by “[NAME], **BEARS walk SOUTH**.”
Followed by “ **STREET”**
Followed by “[NAME], **if you are in pain squeeze my hand two times**.”
Followed by “ **TRUCK**”
Followed by “[NAME], **if you are ok squeeze my hand two times**.”
Followed by “ **BUTTER**”
Followed by “[NAME], **show me two fingers**”
Followed by “ **BOWL**”
Followed by “[NAME], **if SHEEP have WOOL, squeeze my hand”**
Followed by “ **CLOCK**”
Followed by “[NAME], **if TREES are made of GLASS, squeeze my hand**.”
Followed by “ **VIOLIN**”
Followed by “[NAME], **squeeze my hand**.”
Followed by “ **MATERIAL**”
Followed by “ **HISTORY**”
Followed by “ **FORK**”
Followed by “ **ENGINE**”
Followed by “[NAME], **squeeze my hand.”**

(WORD RETRIEVAL TASK)

"Now, we are going to do an exercise where I read you two words. I want you to pay attention to whether one of these words seems to be more familiar to you than the other. If you do have a sense that one seems more familiar to you, tell me that word. If neither word seems more familiar, then please choose one of the words randomly."

Time Start:_____ EEG time:_____

RETRIEVAL LIST A/B

(1)	VIOLIN	**BASKET**
(2)	**EGG**	FORK
(3)	TRUCK	**HAT**
(4)	SOUTH	**NORTH**
(5)	HISTORY	**OFFICE**
(6)	**WATER**	GLASS
(7)	**TABLE**	STREET
(8)	**PENCIL**	BUTTER
(9)	ENGINE	**JOURNAL**
(10)	BOWL	**LAMP**
(11)	**FISH**	SHEEP
(12)	**STOVE**	CLOCK
(13)	**BIRDS**	BEARS
(15)	**INDUSTRY**	MATERIAL
(14)	WOOL	**SEA**
(16)	TREES	**STONES**

RETRIEVAL LIST C/D

(1)	BASKET	**VIOLIN**
(2)	**FORK**	EGG
(3)	HAT	**TRUCK**
(4)	NORTH	**SOUTH**
(5)	OFFICE	**HISTORY**
(6)	**GLASS**	WATER
(7)	**STREET**	TABLE
(8)	**BUTTER**	PENCIL
(9)	JOURNAL	**ENGINE**
(10)	LAMP	**BOWL**
(11)	**SHEEP**	FISH
(12)	**CLOCK**	STOVE
(13)	**BEARS**	BIRDS
(15)	**MATERIAL**	INDUSTRY
(14)	SEA	**WOOL**
(16)	STONES	**TREES**

Number of words identified correctly: _____
Did these words seem familiar to you?

(CAM ICU)

COMMENTS:

Feature 1: Acute Onset or Fluctuating Course

Feature 2: Inattention

SAVEAHAART

Feature 3: Altered Level of Consciousness

RAAS ≠ 0

Feature 4: Disorganized Thinking

Will a stone float on water?

Are there fish in the sea?

Does one pound weigh more than two pounds?

Can you use a hammer to pound a nail?

CAM-ICU POSITIVE:

CAM-ICU NEGATIVE:

CAM ICU

COMMENTS:

Feature 1: Acute Onset or Fluctuating Course

Feature 2: Inattention

SAVEAHAART

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