Electroencephalogram Studies of Induction and Recovery from Sevoflurane-Induced General Anesthesia

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I. BACKGROUND AND SIGNIFICANCE

Sevoflurane is an anesthetic agent with a rapid induction, emergence and recovery profile.[1] Evidence suggests that sevoflurane, similar to other ether derivatives in clinical use, exerts its physiological and behavioral effects by binding at multiple targets in the brain and spinal cord.[2] Action at these targets includes potentiation of γ-Aminobutyric acid ($GABA_A$), glycine and two-pore potassium channels; and inhibition of voltage-gated potassium, N-methyl-D-aspartate (NMDA), muscarinic and nicotinic acetylcholine (ACH), serotonin, and α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) channels.[2-5] Despite detailed characterizations of the molecular and cellular pharmacology of anesthetics, the neural circuit-level mechanisms of general anesthesia-induced unconsciousness are still being actively investigated.[3,4] Extensive work has related in detail the electroencephalogram (EEG) patterns of propofol (2,6-di-isopropylphenol) to its neural circuit mechanisms.[6-12] Propofol primarily acts at $GABA_A$ receptors throughout the brain and spinal cord to enhance inhibition.[3-5,13,14] It also potentiates glycine receptors, and provides inhibition to voltage-gated potassium, ACH, AMPA and Kainate channels.[3-5] Given the diversity of receptor targets, a unitary hypothesis of the neural circuit mechanism underlying anesthesia-induced depression of consciousness does not seem likely. However, clinically, we have observed that sevoflurane induces stereotypical changes in the EEG that appear grossly similar to propofol and that may help relate this anesthetic vapor to its neural circuit mechanisms.

Our previous work provides compelling evidence that unconsciousness under propofol is characterized on the EEG by alpha (8-12 Hz) oscillations that are coherent across the frontal cortex, delta (1-4 Hz) oscillations and high amplitude slow (0.1-1Hz) oscillations.[7,9,11,13,15-17] Intracortical recordings during propofol-induced unconsciousness suggest that local and long-range cortical communication are impeded by temporally and spatially incoherent slow oscillations that exhibit phase-limited spiking.[15] Analysis of the scalp EEG, a readily accessible measure of the average activity in large populations of cortical neurons, has established that propofol induces synchronous frontal alpha oscillations.[7,9-11] Biophysical modeling provides further evidence that propofol induces coherent alpha activity by increasing $GABA_A$ conductance and decay time.[6,12] This increase in $GABA_A$ conductance facilitates involvement of the thalamus in a highly coherent thalamocortical alpha oscillation loop.[6,12] This pathologically coherent frontal alpha oscillation pattern reduces the dimensionality of the thalamocortical network, reducing the ability of the thalamus to project and coordinate exogenous inputs to the neocortex.[12-14]

Coherent alpha oscillations have also been identified in animal studies of the inhaled anesthetics during unconsciousness.[18-20] However, human studies examining this cortical dynamic are non-existent. Given that both sevoflurane and propofol are known to act at $GABA_A$ receptors,[3-5,13,14] studying the EEG patterns elicited by sevoflurane using the novel paradigms we have previously established to study propofol will provide insights on the neural circuit mechanisms of sevoflurane. Previous studies employing EEG-based methods, including spectral analysis, bispectral analysis, auditory evoked potentials and methods for source localization, have been used to compare the conscious and unconscious states under general anesthesia, usually from surgical recordings taken before and after a rapid induction of general anesthesia.[21] However, none of these methods have been used to track, on a moment-to-moment basis, the

transition to unconsciousness during a gradual induction of general anesthesia with an anesthetic vapor, nor the return of consciousness.

We have recently studied the similarities and differences in EEG spectra and coherences between sevoflurane and propofol in data recorded during routine care of patients in the intraoperative setting [publication in review]. We found the following: (i) Similar to propofol-induced frontal alpha oscillations, sevoflurane is characterized by coherent alpha oscillations with similar maximum power and coherence occurring at ~10-12 Hz; (ii) Also similar to propofol, sevoflurane is associated with slow oscillations characterized by similar power and coherence at frequencies < 1 Hz; (iii) In contrast to propofol, sevoflurane is associated with increased power in the theta band. These theta oscillations are coherent across the frontal cortex and may provide additional insight into the neural circuit mechanisms of sevoflurane especially with regards to thalamic dysrhythmia and deafferentation. However, since the EEG recordings we analyzed were obtained solely from frontal channels in a clinical setting, we were unable to perform detailed characterizations of changing behavior and consciousness during controlled induction and emergence, limiting our inferences to a clinically unconscious state. Also, the nature of data (frontal channel only) limited our ability to analyze the cortical dynamics underlying sevoflurane anesthesia-induced unconsciousness.

Therefore, we propose a paradigm to use EEG measurements to study the dynamics of loss of the changes to well-designed sensory or cognitive “probes.” This study will help address the compelling need to replace the current clinical definitions of general anesthesia with neurophysiological and neuroanatomical definitions of the state of general anesthesia. By using principled auditory, and somatosensory asks, results from this study will help establish the relation between EEG changes and loss of consciousness. This may provide insights into the neural circuit mechanisms through which sevoflurane and other ether derivatives induce unconsciousness. The detailed characterization and comprehension of these mechanisms may prove vital for shaping future studies on establishing the neural correlates of consciousness.

II. SPECIFIC AIMS

The specific aims of this study are:

AIM 1: To use high-density EEG recordings to characterize the different brain-states induced by the anesthetic vapor, sevoflurane.

Hypothesis 1a. The onset and stable maintenance of sevoflurane-induced beta oscillations will signify a sedative brain state with a probability of response to auditory and nociceptive stimuli that approaches the awake state.

Hypothesis 1b. The onset and stable maintenance of highly coherent sevoflurane-induced frontal alpha and theta oscillations will signify a brain-state approaching a zero probability of response to auditory and nociceptive stimuli.

Hypothesis 1c. Sevoflurane-induced large slow oscillations (at maximum amplitude) and burst suppression will signify a brain state with a zero probability of response to auditory stimuli.

Hypothesis 1d. Return of consciousness will be preceded by return of gamma oscillations and the loss of large amplitude slow, theta, alpha and beta oscillations.

AIM 2: To use high-density EEG recordings to understand the neural-circuit mechanism of action of ether anesthetics.

Hypothesis 2a. The administration of an N-methyl-D-aspartate receptor antagonist (ketamine) during sevoflurane-induced unconsciousness (putative γ-aminobutyric acid mechanism) will antagonize and significantly diminish the amplitude of the alpha and slow oscillations.

Hypothesis 2b. The administration of an N-methyl-D-aspartate receptor antagonist (ketamine) during sevoflurane-induced unconsciousness (putative γ-aminobutyric acid mechanism) will produce globally non-coherent beta oscillations that are sufficient to maintain a brain state with a zero probability of response to auditory stimuli.
Hypothesis 2c. The administration of an N-methyl-D-aspartate receptor antagonist (ketamine) during sevoflurane-induced unconsciousness (putative γ-aminobutyric acid mechanism) will antagonize the effects of sevoflurane on airway collapsibility by mediated by γ-aminobutyric acid inhibition on the hypoglossal motor neuron.

AIM 3: To use inflammatory profiling of serum to understand the effect of sevoflurane on systemic inflammation
Hypothesis 3a: We hypothesize that sevoflurane-anesthesia will be associated with increased levels of pro-inflammatory cytokines interleukin 6 and TNF-alpha.
Hypothesis 3b: We hypothesize that sevoflurane/ketamine-anesthesia will be associated with decreased levels of pro-inflammatory cytokines interleukin 6 and TNF-alpha.

III. SUBJECT SELECTION

We will select 24 volunteer subjects (male and female) between the ages of 18-50 years. The subjects will be recruited using:

- An announcement of the study distributed through the Partners Public Affairs distribution list.

All study subjects will be American Society of Anesthesiologists (ASA) physical status classification P1. That is, all study subjects will be fit and healthy. A complete medical history will be taken and a complete physical examination will be given to rule out active and chronic medical problems.

Primary Inclusion Criteria for Subjects

- Between the ages of 18 and 50
- Normal body weight and habitus, BMI ≤ 30
- Non-smoker
- American Society of Anesthesiologists (ASA) physical status classification P1

Medical Reasons for Exclusion
Chronic health conditions that will exclude subjects from the study include but are not limited to:

Cardiovascular:	hypertension, myocardial infarction, and coronary artery disease, peripheral vascular disease, arrhythmia, congestive heart failure, valvular disease
Respiratory:	sleep apnea, bronchitis, chronic obstructive pulmonary disease, smoking, shortness of breath
Hepatic:	hepatitis, jaundice, ascites
Neurologic:	seizure, stroke, positive neurologic findings on neurologic examination, multiple sclerosis, Meniere's disease, Parkinson's disease

Gastrointestinal:	esophageal reflux, hiatal hernia, ulcer
Endocrine:	glucose intolerance, diabetes, thyroid disease
Renal:	acute or chronic severe renal insufficiency
Hematologic:	blood dyscrasias, anemia, coagulopathies, on anticoagulant therapy
Musculoskeletal:	prior surgery or trauma to head neck or face, arthritis, personal or family history of malignant hyperthermia
Psychiatric:	claustrophobia, history or treatment for an active psychiatric problem, depression
Reproductive:	pregnancy, breastfeeding
Medications:	regular use of prescription and non-prescription medications expected to affect CNS function
Allergies:	sevoflurane, phenylephrine, glycopyrrolate**,** ketamine, haldol

Remuneration

For successful completion of this protocol, subject remuneration will be $800. If the study subject is unable to complete the entire protocol, pro-rating of this remuneration will be as follows:

- Study subjects who complete the medical screening evaluation but do not begin the anesthesia portion of the study will receive $50.
- Study subjects who complete the medical screening evaluation, and one anesthesia study visit will receive $250.
- Study subjects who complete all study portions will receive $800.

Parking vouchers will be available on study days.

IV. STUDY PROCEDURES

Location:

The screening visit will take place at the White 12 Clinical Research Center.

The anesthesia portion of these studies will take place on White 5 at the Carl Rosow Center for Clinical Research. White 5 was constructed specifically for the performance of research on human subjects receiving the depressant medications used in anesthesia. The research area is built to the same standard as a typical surgical procedure room at MGH with respect to ventilation, temperature control, plumbing and electrical facilities, as well as storage for sterile supplies and linens. There is piped oxygen and air, wall suction and waste anesthetic gas removal. Electrical outlets are supported by the hospital's backup generator. There is a fully-equipped anesthesia machine with an automated record keeper and standard anesthesia monitors. A fully equipped code cart is immediately available, and an emergency call system is connected to the White 3 PACU. The code cart and medications are checked at intervals by personnel from the

OR pharmacy, and the anesthesia equipment is safety checked and maintained by Anesthesia Bioengineering. In short, research subjects receiving anesthetic drugs in this unit are given an identical standard of care as patients in our operating rooms. Any research protocol utilizing this space must first be reviewed by a DACCPM committee specifically created to ensure appropriate standards of anesthetic care and the safety of human subjects.

Screening Visit:
All subjects will complete a screening visit. Study subjects will arrive at the study site to undergo screening to ensure that they meet the basic inclusion/exclusion criteria for the study and give written informed consent. All questions will be explained as needed. All women of childbearing age will be tested for pregnancy. A urine hCG pregnancy test will be performed. If the test performed comes back negative for pregnancy, the subject will be enrolled. If the test performed comes back positive for pregnancy or the subject is breastfeeding, the subject will not be enrolled in the study. If the test performed comes back borderline, we perform another test.

Consent Procedures: Prior to the study, each subject will sign an informed consent form. The investigator obtaining consent will explain in detail the protocol of the study, its purpose and potential benefits to the society. Subjects will be informed that if they feel uncomfortable with the study, they can choose to terminate the study at any time.

Physical Examination: The subjects will be given a standard pre-anesthetic physical examination. Particular attention will be paid to the subject's airway anatomy and neurologic function. Any abnormal findings on physical examination will be reason for exclusion from the study. Abnormal findings will be reported to the subjects and recommendations for medical follow-up will be given as needed.

Screening Tests: A complete blood count, blood glucose level, liver function tests (LFTs), blood urea nitrogen (BUN), and creatinine (Cr) level will be obtained at the initial screening visit. For inclusion into the study protocol, each subject will be required to have a platelet count, blood glucose, BUN levels, Cr levels, white blood cell count, hemoglobin, differential and LFTs within 2 times the upper and lower limit of normal. Any subject who tests positive for any "street drugs" (amphetamine, barbiturates, benzodiazepines, cannabinoids, cocaine, opiates, or phencyclidine) may be excluded from the study. An ECG will also be performed.

Randomization: Visits 1 and 2 will be randomized to determine their order of occurrence: sevoflurane only vs. sevoflurane with ketamine.

Study Visit 1 and 2:
Study subjects will arrive at the study site and confirmation of the study subjects' fasting status (minimum 8 hours) will be made. A urine sample will be obtained for a toxic substance screen and for female study subjects, pregnancy testing.

Blood Draws: For all subjects, we will acquire blood samples during Study Visits 1 and 2. The blood draws taken before and after anesthetic exposure will comprise the following: 1.) approximately 2 ml of whole blood for analysis using the 44-analyte TruCultureMAP. Analytes in this map include interleukin 6 and TNF-alpha and, 2.) approximately 13 ml of blood will be acquired processed into serum and stored for downstream studies and

confirmatory assays of TruCultureMAP results.

An EEG montage with a maximum of 256 channels will be placed on the subject. Standard physiological monitors for anesthesia will be placed including: electrocardiogram, pulse oximeter, and non-invasive blood-pressure cuff. End-tidal CO_2 measurements will be made by way of a capnogram attached to the expiratory port on the breathing circuit. Galvanic skin response will also be measured. A cuff pain delivery device will be attached to the gastrocnemius area of the lower leg. Baseline vital signs will then be taken. A peripheral intravenous line will be placed and an infusion of ringer's lactate or normal saline will be started. Baseline physiologic measurements will be taken.

The study protocol will be divided into 3 segments. These segments are defined in order as: Baseline Recording; Induction and Recovery from Hypnosis; Post-Anesthesia Recovery.

One time before and one time after the study period, a calibrated cuff pain stimuli will be delivered to the gastrocnemius area of the lower leg using a validated cuff pain device titrated to 8-9/10 pain.[22-25] A computer controlled air compressor (Hokanson Rapid Cuff Inflator) will inflate the cuff to a pre-specified pressure, and maintain the pressure at that level. Pressure will be maintained for up to 5-minutes to evaluate brain, cardiovascular, and subjective response to experimental pain. One advantage to using cuff algometry pain is that unlike more superficial methods (e.g. heat pain), cuff pain responses are unaffected by sensitization or desensitization of the skin, indicating that this procedure primarily assesses sensitivity in muscle and other deep tissues.[22]

The following questionnaires will be administered during the baseline and recovery periods to assess pain intensity and quality, measure levels of emotional distress and cognitive state.:

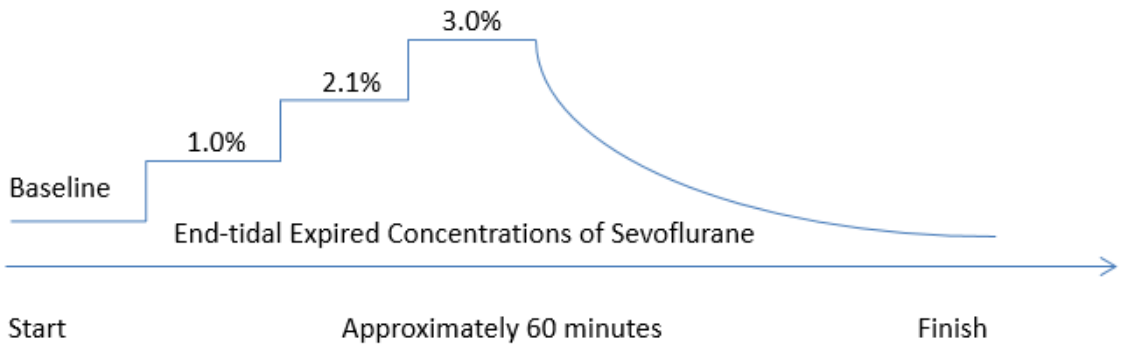
- PROMIS Numeric Rating Scale V1.0 – Pain Intensity 1A;
- PROMIS Scale v2.0 – Neuropathic Pain Quality 5a;
- PROMIS Scale v2.0 – Nociceptive Pain Quality 5a.
- PROMIS Item Bank v.1.0 – Emotional Distress-Anxiety Short Form 8A (modified to ask for symptoms experienced within the past hour);
- PROMIS Item Bank v.1.0 – Meaning and purpose Short Form 8A;
- PROMIS Item Bank v.1.0 – Positive Affect Short Form 15A (modified to ask for symptoms experienced within the past hour);
- The Clinician Administered Dissociative States Scale (CADSS)

In addition, inattention, memory and risk assessment cognitive domains will be evaluated using the following three computer presented neurocognitive tasks respectively: 1) Global Precedence, 2) Operation Span and 3) Columbia Card tasks (https://www.neurobs.com).

Recording of physiological measurements (blood pressure, heart rate, pulse oximetry, end tidal carbon dioxide, and galvanic skin response) will be initiated and maintained from the beginning of the study protocol until the subject is in the post-anesthesia recovery phase.

Induction and Recovery – Sevoflurane Only Protocol: Once the baseline measurements are completed volunteers will receive sevoflurane anesthesia, delivered via a Dräger Fabius Tiro (Telford, PA, USA) anesthesia machine, in high-flow oxygen/air admixture through a secured facemask to approximately achieve the following end tidal concentrations: 1, 2.1 and 3%. The inspired sevoflurane concentration will be adjusted until the desired end tidal sevoflurane concentration is achieved. Each sevoflurane end tidal concentration target will be maintained for approximately 8-12 minutes.

Study Schema – Sevoflurane only

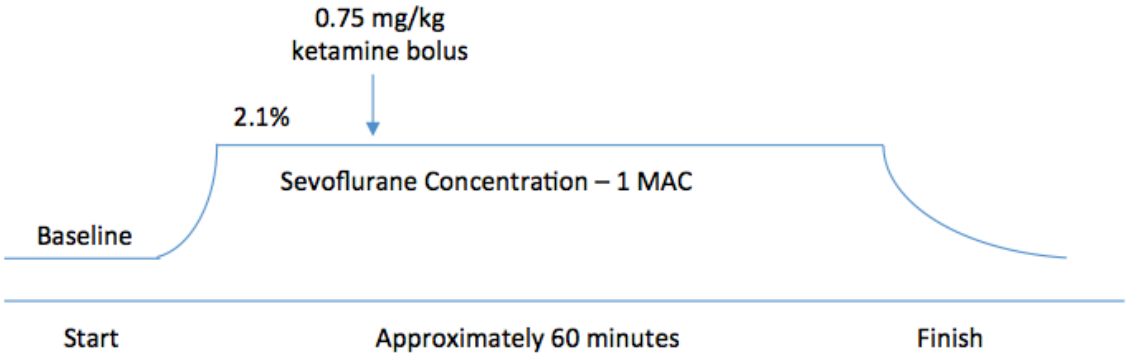


We will target each end-tidal expired concentration of sevoflurane for approximately 8-12 minutes.

Induction and Recovery – Sevoflurane and Ketamine Protocol: Once the baseline measurements are completed, volunteers will receive sevoflurane anesthesia, delivered via a Dräger Fabius Tiro (Telford, PA, USA) anesthesia machine, in high-flow oxygen/air admixture through a secured facemask to approximately achieve the following end tidal concentration: 2.1%. The inspired sevoflurane concentration will be adjusted until the desired end tidal sevoflurane concentration is achieved.

After a stable target concentration of sevoflurane (approximately 1 MAC) has been established for approximately 12 minutes, a 0.75 mg/kg (lean body weight) bolus of ketamine will be administered. The sevoflurane concentration will be held constant for approximately 50 minutes.

Study Schema – Sevoflurane and Ketamine



The total time under sevoflurane will be approximately the same for both protocols.

During the administration of sevoflurane the subject will be assigned to complete the following auditory task:

Auditory task: Subjects will be asked to respond to auditory stimuli via button-press jittered at random between 4 and 8 second intervals in order to resolve the precise period at which loss and recovery of consciousness/responsiveness occurred at a fine temporal scale. The auditory stimulus will consist of two components, a train of “clicks” and the command “click the mouse”. Subjects will be asked to respond by pressing the mouse in the hand. The sound intensity for all stimuli will not exceed 85 dB sound pressure level (SPL), in accordance with National Institute for Occupational Safety and Health guidelines for occupational noise exposure (U.S. Department of Health and Human Services and National Institute for Occupational Safety and Health, 1998).

Precautions: An experienced board certified anesthesiologist, who is not involved in data acquisition or other study functions will monitor and care for the subject according to the standards for delivery of anesthesia at this institution. The White 5 research unit is equipped with an anesthesia machine, and physiologic data are collected using a Metavision electronic anesthesia record. This research unit has been specifically designed and equipped according to MGH standards and Massachusetts regulations as an anesthetizing location, and it has been approved for this purpose by the Clinical Practices and Safety Committee of the MGH Department of Anesthesia, Critical Care and Pain Medicine. If more care is needed in the judgment of the supervising clinician, the subject may be transferred to the MGH White 3 PACU.

Blood Pressure Management: Decreases in systemic arterial blood pressure are a known, expected side effect of sevoflurane. This is because sevoflurane depresses myocardial contractility and decreases systemic vascular resistance. This is manifested clinically as hypotension, defined as a decrease in mean arterial pressure (MAP) of 15%. Significant decreases in blood pressure caused by sevoflurane will be treated with intravenous phenylephrine. Phenylephrine is a synthetic sympathomimetic drug that selectively stimulates alpha-1 adrenergic receptors. In its clinical use, there is no appreciable effect on beta-adrenergic receptors. Resulting venoconstriction is greater than arterial constriction. Using an alpha-adrenergic agonist such as phenylephrine to treat hypotension is advantageous because this counters the sevoflurane-induced decrease in systemic vascular resistance. A particular advantage of phenylephrine is that it is usually administered by intravenous infusion and can therefore be easily titrated to effect.

The phenylephrine infusion will be prepared according to usual practices of the MGH Department of Anesthesia and Critical Care: 1 ml of 1% phenylephrine (20 mg/ml) will be diluted in 250 ml normal saline or lactated ringer solution (final concentration 80 ug/ml). The 251 ml bag will be labeled using a standard, phenylephrine identification sticker confirming the contents, concentration and time and date of preparation of the phenylephrine mixture according to usual Department practices. The intravenous phenylephrine infusion will be titrated using a standard clinical infusion pump to maintain the mean arterial pressure within 15% of the study subject's baseline measurement. The infusion will be titrated and will be delivered within the range of 10 ug/min – 100 ug/min (15 ml/hr – 150 ml/hr).

Glycopyrrolate may also be used to treat changes in blood pressure. Glycopyrrolate is advantageous because this counters the decrease in heart rate without interfering with study measurements by crossing the blood brain barrier. Another particular advantage of glycopyrrolate is that it is usually administered by intravenous infusion and can therefore be easily titrated to effect.

Delirium Management: We have carefully selected ketamine 0.75mg/kg based on evidence that this dose is safe when used alone or in combination with sevoflurane. This dose is within the range that is typically administered in the MGH operating rooms as an anesthetic adjunct to help limit the exposure to narcotics. This dose has also been used in clinical research studies of depression at MGH (0.75mg/hr over 45 minutes; N-methyl-D-aspartate Antagonist (Ketamine). Thus, the administration of an adjunctive dose of ketamine as a bolus, with emergence from anesthesia approximately 60 minutes afterwards should constitute minimal delirium risk. However, in the event the subject is not oriented to space and place, attempting to pull out intravenous lines, is adjudged to constitute a risk to self and/or study staff, haloperidol 1-2 mg will be administered by one of the study anesthesiologists.

Airway Management: Induction of general anesthesia will cause predictable changes in respiratory function. General anesthetic agents, including sevoflurane, can produce apnea after the loss of consciousness. While the study subject is awake, oxygenation and ventilation will be spontaneous. The standard clinical approach for airway management in surgical patients is for the anesthesiologist to assist oxygenation and ventilation after the loss of consciousness. With progressive loss of consciousness, the study subject will hypoventilate and become apneic. The staff anesthesiologist will use standard airway maneuvers to maintain normal oxygenation and ventilation. These maneuvers include a jaw and chin thrust and assisted ventilation with a mask and bag as part of a circle circuit. Ventilation will be manually assisted as needed. At all times, the inspired oxygen concentration and expired carbon dioxide waveform and partial pressure will be monitored continuously. The minimum inspired oxygen concentration will be 30%. Thus, an anesthesiologist may manually ventilate the subject. The end-tidal (capnogram) carbon dioxide levels will be maintained within 10% of the baseline values. Manually assisted oxygenation and ventilation will continue until the subject recovers spontaneous ventilation.

Post-Anesthesia Recovery: Following Induction and Recovery, the subject will be monitored for post-anesthesia recovery for up to 1 to 2 hours as is done for patients following ambulatory surgery. The subject will be discharged based on established MGH Department of Anesthesia practices for discharge from the hospital following ambulatory surgery. The subject must have stable vital signs, i.e., within 20% of pre-study values, be able to respond appropriately to normal commands, be pain-free, be free from any nausea and vomiting, and have no bleeding from the intravenous site. Subjects must be able to walk unassisted and have an accompanying adult to escort them home. Subjects will be advised not to return to work for 12 hours and be advised against driving or operating heavy equipment for 24 hours.

VI. BIOSTATISTICAL ANALYSIS

We are using an endpoint of performance score on the Global Precedence task to guide sample size determination. Our null hypothesis is the average within subject difference in performance score for sevoflurane compared to sevoflurane/ketamine is zero. Our

alternative hypothesis is the average within subject difference in performance score for sevoflurane compared to sevoflurane/ketamine is 3. If we assume that the variance in performance score is 9, a type I error of 0.05 and a minimum power of 0.90 for a one-sided z-test, the sample size determinations for our study is 9. Therefore, we should detect a clinically relevant improvement in performance score if we successfully enroll only 9 subjects. To account for data unanticipated events that may lead to poor data quality or data loss, we are proposing to enroll 12 subjects.

EEG acquisition Preprocessing: Continuous EEG will be recorded with up to 256 channels. All data will be stored for subsequent off-line analysis. We will apply an anti-aliasing filter and down-sampled the EEG data to 500 or 250 Hz before analysis. Bandpass filtering of the acquired signal will vary depending upon the features of the EEG data of interest. EEG signals will be remontaged to a nearest-neighbor Laplacian reference, using distances along the scalp surface to weigh neighboring electrode contributions. Trigger signals will be sent from the stimulus delivery system to the EEG recording system to tag the stimulus events for subsequent binning, temporal epoching and averaging of EEG data into the relevant evoked potential averages according to stimulus type and experimental condition.

Video recording to assess level of arousal: Video of the study subject may be recorded during the baseline and induction and recovery from hypnosis periods to assess the study subject's level of arousal. In particular, from clinical experience and previous studies, we note that subjects will exhibit varying levels of arousal after the loss of consciousness, visible in terms of spontaneous movement, which can be recorded on video and then correlated with the EEG. Permission to record video for these data analysis purposes will be obtained from the main research consent form. The video recordings will be stored in a locked file in the investigators office.

Behavioral Analysis: The probability of response to the click and verbal stimuli and the difference in probability of response will be estimated by using Bayesian Monte Carlo methods to fit a state-space model to these data. To perform group-level analyses, we will align the behavioral data across subjects with respect to each subject's LOC time for induction and with respect to each subject's ROC time for emergence. We will then pool the responses within bins. The pooled data will be used to estimate group-level probabilities of response using the state-space model.

Spectral Analysis: We will compute spectrograms for all subjects using the multitaper method. We will also compute group-level baseline-normalized spectrograms for induction and emergence by taking the median across subjects with the data aligned to the LOC and ROC time points, respectively. To determine if the group-level spectrogram is significantly greater than baseline, we will perform a two-group-test as implemented in chronux.

Eigenvalue and Modal Projection Analyses: We will perform an eigenvector decomposition analysis of the cross-spectral matrix to identify the principal modes of oscillation in the conscious and unconscious states and to analyze how activity within these principal modes change through time. We will estimate the cross-spectral matrices $P_{baseline}(f)$ and $P_{unconscious}(f)$ at each frequency f using the multitaper method using data from the full baseline period, and segments of at least 5 min extending from LOC to ROC, respectively, for each subject. An eigenvalue decomposition of the median cross-spectral matrix at each frequency will be performed. Each eigenvector describes a

coherent spatial distribution or mode of oscillation, and the corresponding eigenvalue quantifies the power in this mode. We then use modal projection analysis to characterize how power within principal modes change as a function of time. A permutation-based procedure will be performed to assess statistical significance for the modal projection analysis.

Phase–Amplitude Modulation Analysis. We will analyze the relationship between low-frequency phase (0.1–1 Hz) and alpha/beta (8–14 Hz) amplitude by calculating a phase–amplitude histogram. Bandpass filters will be applied to construct narrow-band slow and alpha/beta signals. We will then apply the Hilbert transform to each signal and compute the low-frequency oscillation phase and alpha oscillation amplitude. To construct the histogram, we will assign each temporal sample to one of 18 equally spaced phase bins based on the value of low-frequency oscillation phase, averaging over 2-min epochs. The histogram in each phase bin is the average of alpha oscillation amplitude for all samples within the bin, normalized by the average of alpha oscillation amplitude over the entire 2-min epoch. To assess statistical significance for the histogram, a permutation test will be performed.

Anticipated Findings: Changes in EEG recordings to auditory, somatosensory, may aid principled approaches to brain state monitoring and targeting during general anesthesia.

VII. RISKS AND DISCOMFORTS

Sevoflurane risks: The risks involved in the administration of sevoflurane include, nausea, vomiting and coughing. Other possible side effects of sevoflurane are increased salivation, fever, and tingling in the extremities or hepatic injury. 5% to 9% of patients experience changes in heart rhythm, agitation, difficulty breathing, decreases in blood pressure, shivering and tiredness. Study subject hemodynamic parameters will be continuously monitored to ensure that appropriate medical intervention will be instituted for any clinically significant hypotensive or bradycardic episodes.

Phenylephrine risks: Risks involved in the administration of phenylephrine include hypertension and bradycardia.

Glycopyrrolate risks: Risks involved in the administration of glycopyrrolate include hypertension and tachycardia.

Ketamine risks: Possible side effects reported during ketamine infusion include an increase in heart rate and blood pressure, arrhythmia, increased salivation, increased bronchial secretions, horizontal nystagmus, euphoria, and hallucinations. Any acute changes in physiology, such as changes in heart rate or blood pressure, will be treated using standard anesthesiology practices. Rare side effects are allergic reactions (skin rash), pain at the site of injection, increased intraocular pressure, ulcerations and inflammation in the bladder (reported in ketamine abusers).

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

Risks of Intravenous Line (IV): Risks involved include bruising, infection, lightheadedness, and fainting.

Hearing risks: Presented auditory stimuli will not exceed 85 dB SPL, as recommended by the National Institute for Occupational Safety and Health, and present minimal risk of hearing loss (U.S.Department of Health and Human Services and National Institute for Occupational Safety and Health, 1998).

Risks associated with cuff pain stimuli: Risks of cuff pain include mild transient bruising associated with inflation of the cuff. In our experience, this is infrequent (< 5 % of cases).In fact, cuff pain has been well tolerated by both healthy adults and chronic pain patients, and according to our data, the pain intensity has been found to remain within stable limits for the duration of the scan run (e.g. 1st 2min: 35.9±12.4; 2nd 2min: 42.9±14.0; final 2min: 43.4±21.6, on a scale of 0-100). Our previous experience has also demonstrated that pain ratings go down to nil within several seconds of stimulus termination. A simple button press can rapidly deflate the cuff, ensuring subject safety.

VIII. Potential Benefits

There are no direct benefits to the individual subjects involved in this study. Sevoflurane- and/or sevoflurane plus ketamine-induced EEG dynamics may aid principled approaches to brain state monitoring and targeting during general anesthesia. They may also lend insights into anti-nociceptive or pain mechanisms.

IX. Monitoring and Quality Assurance

No identifiers other than study ID's will be included in the dataset. EEG data will be stored for later off-line analysis. Safety monitoring will include immediately available cardiopulmonary resuscitation cart, oxygen, ambu bag and defibrillator for use in need of an emergency. A fully functional O_2 delivery system monitors, and a fully stocked anesthetic cart with equipment for airway management will also be available.

One clinical board certified level anesthesiologist who is not taking part in the execution of the study protocol will be present at all times and responsible only for the medical management of the patient. This anesthesiologist will have the ability to halt the study at any point if he/she determines that this would be in the best interest of the subject. Prior to the start of the study it will be verbally reiterated what each person is responsible to execute if an emergency should occur as noted:

1. Clinical Anesthesiologist – will call and run code
2. Study Anesthesiologist – will assist with code, call White 3 PACU if needed
3. Clinical Coordinator – Keep time and notes of all events

Study Stopping Criteria:
Prior to study initiation, the subject will be instructed to alert the study staff to the occurrence of pain, discomfort or anxiety. The study physician will interrupt the study and attempt to alleviate the source of pain, discomfort, or anxiety.

To eliminate any ambiguity with hand signals, the subject will be instructed to give a "thumbs up" for an affirmative answer, and to wiggle his/ her index finger back and forth to give a negative answer. No more than 2 separate incidents will be allowed before the study is aborted. For any single incident, the investigators will spend no more than 20 minutes to alleviate pain or discomfort before the study is aborted. At any time prior to the 20-minute or 2-alert limits, the clinical anesthesiologist can require the study anesthesiologist to abort the study if, in his or her judgment, the safety of the subject is

in question. Also, the clinical anesthesiologist can also require the study anesthesiologist to abort the study at any time for any subject safety-related concerns.

X. REFERENCES

1. Eger EI, 2nd. New inhaled anesthetics. Anesthesiology 1994;80:906-22.
2. Campagna JA, Miller KW, Forman SA. Mechanisms of actions of inhaled anesthetics. N Engl J Med 2003;348:2110-24.
3. Alkire MT, Hudetz AG, Tononi G. Consciousness and anesthesia. Science 2008;322:876-80.
4. Franks NP. General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. Nat Rev Neurosci 2008;9:370-86.
5. Rudolph U, Antkowiak B. Molecular and neuronal substrates for general anaesthetics. Nat Rev Neurosci 2004;5:709-20.
6. Vijayan S, Ching S, Purdon PL, Brown EN, Kopell NJ. Thalamocortical mechanisms for the anteriorization of alpha rhythms during propofol-induced unconsciousness. The Journal of neuroscience : the official journal of the Society for Neuroscience 2013;33:11070-5.
7. Purdon PL, Pierce ET, Mukamel EA, et al. Electroencephalogram signatures of loss and recovery of consciousness from propofol. Proceedings of the National Academy of Sciences of the United States of America 2013;110:E1142-51.
8. Chen Z, Purdon PL, Harrell G, et al. Dynamic assessment of baroreflex control of heart rate during induction of propofol anesthesia using a point process method. Ann Biomed Eng 2011;39:260-76.
9. Cimenser A, Purdon PL, Pierce ET, et al. Tracking brain states under general anesthesia by using global coherence analysis. Proceedings of the National Academy of Sciences of the United States of America 2011;108:8832-7.
10. Murphy M, Bruno MA, Riedner BA, et al. Propofol anesthesia and sleep: a high-density EEG study. Sleep 2011;34:283-91A.
11. Supp GG, Siegel M, Hipp JF, Engel AK. Cortical hypersynchrony predicts breakdown of sensory processing during loss of consciousness. Current biology : CB 2011;21:1988-93.
12. Ching S, Cimenser A, Purdon PL, Brown EN, Kopell NJ. Thalamocortical model for a propofol-induced alpha-rhythm associated with loss of consciousness. Proceedings of the National Academy of Sciences of the United States of America 2010;107:22665-70.
13. Brown EN, Lydic R, Schiff ND. General anesthesia, sleep, and coma. The New England journal of medicine 2010;363:2638-50.
14. Brown EN, Purdon PL, Van Dort CJ. General anesthesia and altered states of arousal: a systems neuroscience analysis. Annual review of neuroscience 2011;34:601-28.
15. Lewis LD, Weiner VS, Mukamel EA, et al. Rapid fragmentation of neuronal networks at the onset of propofol-induced unconsciousness. Proceedings of the National Academy of Sciences of the United States of America 2012;109:E3377-86.
16. Feshchenko VA, Veselis RA, Reinsel RA. Propofol-induced alpha rhythm. Neuropsychobiology 2004;50:257-66.
17. Gugino LD, Chabot RJ, Prichep LS, John ER, Formanek V, Aglio LS. Quantitative EEG changes associated with loss and return of consciousness in healthy adult volunteers anaesthetized with propofol or sevoflurane. Br J Anaesth 2001;87:421-8.
18. Li D, Voss LJ, Sleigh JW, Li X. Effects of volatile anesthetic agents on cerebral cortical synchronization in sheep. Anesthesiology 2013;119:81-8.

19. Kreuzer M, Hentschke H, Antkowiak B, Schwarz C, Kochs EF, Schneider G. Cross-approximate entropy of cortical local field potentials quantifies effects of anesthesia--a pilot study in rats. BMC neuroscience 2010;11:122.
20. Voss LJ, Ludbrook G, Grant C, Sleigh JW, Barnard JP. Cerebral cortical effects of desflurane in sheep: comparison with isoflurane, sevoflurane and enflurane. Acta anaesthesiologica Scandinavica 2006;50:313-9.
21. John ER, Prichep LS, Kox W, et al. Invariant reversible QEEG effects of anesthetics. Conscious Cogn 2001;10:165-83.
22. Polianskis R, Graven-Nielsen T, Arendt-Nielsen L. Computer-controlled pneumatic pressure algometry--a new technique for quantitative sensory testing. Eur J Pain 2001;5:267-77.
23. Polianskis R, Graven-Nielsen T, Arendt-Nielsen L. Pressure-pain function in desensitized and hypersensitized muscle and skin assessed by cuff algometry. J Pain 2002;3:28-37.
24. Polianskis R, Graven-Nielsen T, Arendt-Nielsen L. Spatial and temporal aspects of deep tissue pain assessed by cuff algometry. Pain 2002;100:19-26.
25. Polianskis R, Graven-Nielsen T, Arendt-Nielsen L. Modality-specific facilitation and adaptation to painful tonic stimulation in humans. Eur J Pain 2002;6:475-84.
26. Harms MP, Melcher JR. Sound repetition rate in the human auditory pathway: representations in the waveshape and amplitude of fMRI activation. Journal of neurophysiology 2002;88:1433-50.
27. Hari R, Lounasmaa OV. Recording and interpretation of cerebral magnetic fields. Science 1989;244:432-6.
28. Liu AK, Dale AM, Belliveau JW. Monte Carlo simulation studies of EEG and MEG localization accuracy. Hum Brain Mapp 2002;16:47-62.
29. Oostendorp TF, van Oosterom A. Source parameter estimation in inhomogeneous volume conductors of arbitrary shape. IEEE Trans Biomed Eng 1989;36:382-91.
30. Hamalainen M, Hari R, Ilmoniemi RJ, Knuutila J, Lounasmaa OV. Magnetoencephalography-- theory, instrumentation, and applications to noninvasive studies of the working human brain. Reviews of Modern Physics 1993;65:413-97.