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Electroencephalogram Studies of Induction and Recovery from Propofol Induced General Anesthesia

Principal Investigator: Emery N. Brown, M.D., Ph.D.

Co-Investigators: Patrick Purdon, Ph.D.; Giorgio Bonmassar, Ph.D.; John Walsh, M.D.; Priscilla G. Harrell, M.D.; Jean Kwo, M.D.; Matti Hämäläinen, Ph.D.; Eric T. Pierce, M.D., Ph.D; Andres F. Salazar Gomez, B.A., Kara J. Pavone, B.S.

BACKGROUND AND SIGNIFICANCE

The general aim of this study is to localize and track changes in brain activity during loss and recovery of consciousness induced by propofol using source localization from electroencephalogram (EEG) recordings analyzed with spectral methods, source localization techniques and event-related potentials (ERP).

Each day in the United States, more than 50,000 patients receive general anesthesia for surgical procedures (Barash et al., 1997). General anesthesia and conscious sedation are also widely used for non-surgical interventions in critical care, radiology, obstetrics, pediatrics, gastroenterology, psychiatry and dentistry. The state of general anesthesia has four characteristics defined in clinical terms. These are hypnosis, amnesia, analgesia and lack of movement (Barash et al., 1997). In anesthesiology, the terms lack of awareness, hypnosis and loss of consciousness are used synonymously. Since World War II there have been significant improvements in the safety of anesthetic drugs, systems for their delivery, and in physiological monitoring systems. Nevertheless, the mechanisms by which anesthetic drugs induce the state of general anesthesia remain a mystery to modern medicine.

An important consequence of not knowing how anesthetic drugs work is that anesthesia-related morbidity is a significant medical problem. This is because all anesthetic agents have profound physiologic effects at sites other than their intended targets in the brain and central nervous system. These side-effects are at best undesirable, but frequently they can be disabling and, in some cases, fatal. These include nausea, vomiting, respiratory depression and abnormal heart rhythms. Post-operative recall is also an important source of anesthesia-related morbidity whose sequelae include sleep disturbances, dreams, nightmares, flashbacks and anxiety, as well as post-traumatic stress disorder (Ghoneim and Block, 1997). For many patients, concern for the possibility of post-operative recall can also be a source of pre-operative anxiety.

There is, therefore, a compelling need to replace the current clinical definitions of general anesthesia with neurophysiological and neuroanatomical definitions of the state of general anesthesia. This means identifying the sites in the brain and central nervous system at which anesthetic drugs are required to act to induce the state of general anesthesia and defining the time course of the actions at those sites. Defining accurately the state of general anesthesia should reduce anesthesia morbidity and mortality by leading to the development of more site-specific anesthetic drugs, and by developing

more neurophysiologically-based methods for monitoring the anesthetic state during surgery.

Mechanisms of Action of Propofol

Most general anesthesia is initiated by administering a potent hypnotic agent that induces loss of consciousness within 20 to 60 seconds. One of the most frequently used hypnotic agents is propofol (2, 6-diisopropylphenol). The mechanisms through which it induces loss of consciousness are not understood. Neurophysiologic studies suggest putative sites of action in both the cortex and midbrain (Koblin, 1994;Rampil et al., 1993). Propofol is a known GABA-A agonist that is postulated to act on GABA-A receptors at the level of the midbrain reticular activating formation and its connections with the hippocampus, hypothalamus, basal ganglia, and medial thalamus (Hales and Lambert, 1991;Hara et al., 1993;Yate et al., 1986). Cerebral blood flow, metabolism and positron emission tomography studies show that propofol acts at sites in the midbrain and in the cortex (Alkire et al., 1995;Cavazzuti et al., 1991;Dam et al., 1990;Pinaud et al., 1990). These apparently precise statements about how this anesthetic drug acts in the brain still does not define exactly the time course through which actions at these receptor sites in these brain regions induces loss of consciousness and how cessation of these actions lead to recovery of consciousness.

Clinical Monitoring Loss of Consciousness Under General Anesthesia

Despite the benefits from the technological advances in anesthetic delivery and monitoring systems and the growing body of information on the molecular mechanisms of anesthetic actions, most anesthesiologists monitor the depth of anesthesia by following clinical variables that are easy to observe. The primary physiological indicators used to establish the adequacy of anesthesia are loss of response to verbal and tactile stimuli, lash reflex, heart rate, blood pressure, and pupil size.

Electroencephalogram Correlates of Loss of Consciousness Under General Anesthesia

It is well established that changes in neurophysiological recordings such as electroencephalogram (EEG) provide a reliable, empirical characterization of anesthesia-induced loss of consciousness (Kearse, Jr. et al., 1992;Pockett, 1999). The characteristic patterns of EEG changes seen with anesthesia-induced loss of consciousness are well documented and are used regularly as part of protocols to monitor the integrity of brain function during surgeries where cerebral blood flow may be compromised (Kearse, Jr. et al., 1992). The EEG changes observed with induction of propofol anesthesia closely resemble the changes seen in the alpha range (15 to 30 Hz) with barbiturates (Forrest et al., 1994;Pockett, 1999;Reddy et al., 1993). Studies evaluating the mechanisms for generating the normal α EEG activity in awake animals and α frequency EEG changes in animals anesthetized with barbiturates have demonstrated coherent electrical activity between sites within the cortex as well as between sites in the cortex and the midbrain (Magni et al., 1959). Various spectral techniques including power spectral analysis (Rampil, 1998), the spectral index (Rampil and Matteo, 1987) and normalized region-specific EEG pattern changes (John and Prichep, 2005) have been associated with loss of consciousness. For example, with loss of consciousness, there is an increase in alpha

power in the spectrum, from anterior to posterior whereas the reverse occurs with return of consciousness. Changes in coherence in EEG activity in the gamma range (60 to 80 Hz) between anterior and posterior brain regions have been related to changes in level of consciousness (John and Prichep, 2005). In particular, it has been demonstrated that with loss of consciousness there is a loss in coherence in the gamma frequency between the frontal and occipital electrodes and with return of consciousness the coherence is reestablished.

The coherence patterns measured from scalp EEG have been further analyzed using bispectral analysis. Bispectral analysis determines both linear and nonlinear components in the spectrum and quantifies between frequency phase-coupling in the EEG signals (Dumermuth et al., 1971). A bispectral index (BIS) has been developed. BIS is a dimensionless number from 0 to 100 computed from bispectral analysis of a patient's EEG. BIS is designed to correlate in real-time with the hypnotic effects of commonly used anesthetic drugs (Glass et al., 1997). An index value of 100 means a subject is fully conscious and capable of actively processing information. A value of less than 20 corresponds to the subject's EEG showing significant periods of burst-suppression - an EEG pattern consistent with the profoundest level of anesthesia-induced hypnosis. The changes in BIS during infusion of hypnotics and synthetic opioids have been systematically studied and correlated with the degree of sedation and level of consciousness (Kearse, Jr. et al., 1994). How values of the BIS relate to activity in specific brain regions is unknown.

Auditory evoked potentials (AEPs) are another type of neurophysiological recording whose changes can depict anesthesia-induced loss of consciousness (Schraag et al., 1999). AEPs are generated in response to auditory stimuli, such as a short "click" or tone. The AEP waveforms are typically very small in comparison to EEG, on the order of 10 μV, so several-hundred such waveforms must be averaged in order to produce an interpretable result. Midlatency AEPs (MLAEP) occur from 10 to 100 ms post-stimulus, are reduced in amplitude under propofol anesthesia, (Pockett, 1999) and have been used as a measure of anesthetic depth during feedback control of anesthesia (Huang et al., 1999). MLAEPs are thought to come from both cortical and sub-cortical generators (Pockett, 1999)and have been linked to auditory perceptual processing (de Beer et al., 1996). Hence, MLAEPs provide a means of actively "probing" a subject's sensory and perceptual state while under anesthesia.

EEG source localization methods have been used in several applications to infer locations in the brain that are responsible for electrical activity (Hamalainen et al., 1993). This is a challenging task because it requires one to compute from the EEG measures made at the scalp the most likely sources in the brain of the observed electrical activity. The electrical activity recorded on the scalp does not show the location of neuronal activity within the brain due to spreading and attenuation of current sources within the head (Hamalainen et al., 1993) because there are infinitely many ways to produce an observed EEG pattern from a given set of EEG sources in the brain. Source localization methods are used to estimate the locations of the sources responsible for the observed activity by assuming a forward model (Zani and Proverbio, 2002) and an algorithm to compute the inverse

solution (Hamalainen et al., 1993). The forward model defines how the electrical activity in the sources propagates to the surface based on assumptions about the geometry of head, number of sources and the different conductivities of the brain, skull and scalp. Solving the inverse problem entails using a statistical criterion function such as least squares along with the assumed forward model to determine the most likely location and magnitude of the sources given the EEG measurements. EEG is a useful tool in source localization because it provides excellent temporal resolution for relating when neural events occur in the brain. MRI provides excellent spatial resolution, indicating where activity takes place in the brain, and can be used to construct more realistic head models for the *forward problem*. EEG source localization can be co-registered with anatomic MRI scans to provide enhanced localization of changes in neuronal activity over time with changes in depth of anesthesia. EEG source localization methods have been recently used without a concurrent MRI anatomic scan to analyze EEG activity in different anesthetic states (John and Prichep, 2005).

Previous studies employing these 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 (John et al., 2001). 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, nor the return of consciousness during a gradual recovery from general anesthesia.

Memory Formation Under General Anesthesia

As stated above, the state of general anesthesia should include amnesia. Studies designed to investigate memory formation under general anesthesia have focused primarily on detecting evidence of post-operative recall. Post-operative recall is thought of in terms of *explicit* and *implicit* memories. With *explicit* memory, the patient is able to consciously recall events experienced during surgery. In contrast, *implicit* memory results in changes in behavior or cognition without conscious recall of the intra-operative events precipitating them. Rates of *implicit* recall during surgical anesthesia have been estimated using retrospective priming studies (Ghoneim and Block, 1997) (Block et al., 1991;Bonebakker et al., 1996). An example of a typical priming study is the "word-stem completion" experiment (Block et al., 1991;Bonebakker et al., 1996). In these experiments, surgical anesthesia patients are presented a large list of tape-recorded words while at a plane of surgical anesthesia. After recovery from anesthesia, the study subjects are presented a list of word-stems that they complete with the first word that comes to mind. For instance, if one were presented the word-stem "CON", one might respond with "CON-STITUTION". Evidence of implicit memory is observed if the stem-completed words show a significant correlation with those presented during anesthesia. One weakness of such retrospective priming studies is that they have low sensitivity, requiring large sample sizes of 70 to 80 study subjects to detect an effect (Block et al., 1991;Bonebakker et al., 1996).

Summary

The time course of changes in neuronal activity associated with gradual induction of and gradual recovery from general anesthesia should be observable using EEG measurements. Furthermore, induction of and recovery from general anesthesia should induce changes in the neuronal responses to simple auditory stimuli, somatosensory stimuli or cognitive tasks. Hence, it should be possible to observe the effects of anesthesia by observing the changes in EEG to well-designed sensory or cognitive "probes." In particular, it should be possible to visualize the influence of propofol on sensory systems by observing changes in MLAEPs during auditory tasks. The relation between EEG changes and loss of consciousness can be established by observing recorded EEG measurements in subjects undergoing anesthesia induction and recovery during these auditory, somatosensory or semantic priming tasks. Therefore, we propose a paradigm to use EEG measurements to study the dynamics of loss of consciousness, auditory processing, sensation, and memory under general anesthesia induced with propofol.

SPECIFIC AIMS

The specific aims of this study are:

Specific Aim 1: Test the hypothesis that we can characterize the relationship between changes of function in the auditory system and loss of consciousness during induction and recovery from propofol general anesthesia using EEG recordings.

Specific Aim 2: Test the hypothesis that we can characterize the relationship between changes of function in the somatosensory system and loss of consciousness during induction and recovery from propofol general anesthesia using EEG recordings.

Specific Aim 3: Test the hypothesis that we can characterize the relationship between changes of function in cognitive systems subserving implicit memory formation and loss of consciousness during induction and recovery from propofol general anesthesia using EEG recordings.

The EEG data in each of the three specific aims will be analyzed using spectral methods, source localization, and event-related potentials.

These studies will take place at the Mallinckrodt General Clinical Research Center (GCRC) Biomedical Imaging Core at Charlestown Navy Yard. The MRI brain scans will also take place at the MRI scanning facility at the Charlestown Navy Yard.

SUBJECT RECRUITMENT

For each specific aim, we will select a group of 20 young healthy, non-smoking male and female volunteers, ages 18 to 36 with normal body weight and body habitus. Thus, 60 study subjects will be chosen to perform an assigned paradigm while receiving propofol general anesthesia. We will also have a group of 20 study subjects that will establish control data for the auditory, cognitive, and somatosensory modalities without the delivery of anesthesia. There will be 80 study subjects in total. The subjects will be recruited using: 1) flyers distributed to the college campuses in the greater Boston area; 2) an announcement of the study distributed through the Partners Public Affairs

distribution list; 3) an announcement placed in the Health/ Science section of the Boston Globe, Boston Herald, Craigslist.com and Boston Metro (see attached announcements); 4) via the MGH Research Study Volunteer Program and; 5) via the website describing the study:http://www2.massgeneral.org/anesthesia/index.aspx?page=research_pain&subpage=statistics3

SUBJECT SELECTION

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.

Medical History. Chronic health conditions that will exclude subjects from the study include but are not limited to:

- Cardiovascular: hypertension, myocardial infarction, coronary artery disease, peripheral vascular disease, arrhythmia, congestive heart failure, valvular disease.
- Respiratory: asthma, sleep apnea, bronchitis, chronic obstructive pulmonary disease, smoking, shortness of breath.
- Hepatic: hepatitis, jaundice.
- Neurologic: seizure, stroke, positive neurologic findings on neurologic examination, multiple sclerosis, Meniere's disease, Parkinson's disease.
- Gastrointestinal: esophageal reflux, hiatal hernia, ulcer.
- Endocrine: diabetes, thyroid disease.
- Hematologic: blood dyscrasias, anemia, coagulopathies.
- Musculoskeletal: prior surgery or trauma to head neck or face, arthritis, personal or family history of malignant hyperthermia.
- Psychiatric: claustrophobia, history or treatment for an active psychiatric problem, depression.
- Reproductive: pregnancy, breast-feeding.
- Medications: regular use of prescription and non-prescription medications expected to affect CNS function.

- Allergies: bisulfite, eggs or egg products, latex, propofol, soybeans, soybean oil, lidocaine, or phenylephrine.

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. Each subject will submit a urine specimen for a toxic substance screen both at the initial examination and on the day of the study. Subjects will be required to take nothing by mouth for 8 hours prior to the start of the study, and no sedative drugs 48 hours prior to the start of the study. Female subjects will be given a pregnancy test at the initial examination and immediately prior to the start of the study. Positive toxicity screening or positive pregnancy testing will be reasons for exclusion from the study. An electrocardiogram (ECG) will be obtained. Hearing level tests will be performed. Blood sampling will be performed to evaluate hemoglobin level. Hemoglobin must be greater than 12.5 g/dl for inclusion in the study.

Other Reasons for Exclusion. High magnetic fields may pose a serious health hazard to subjects with implanted ferromagnetic objects. Every participant in this study will be screened for implanted ferromagnetic objects before they are enrolled and will provide written responses to a questionnaire to screen for implanted ferromagnetic objects before entering the high magnetic field shielded room. A copy of this MRI exclusion questionnaire is appended to this document. Subjects with the following conditions/diseases will be excluded from the study:

- History of head trauma
- Surgical aneurysm clips
- Cardiac pacemaker
- Prosthetic heart valve
- Neurostimulator
- Implanted pumps
- Cochlear implants
- Metal rods, plates
- Screws
- Intrauterine device
- Hearing aid
- Dentures (which might create NMR artifacts)
- Metal injury to eyes

SUBJECT ENROLLMENT

Prior to the study, each subject will sign witnessed, informed consent for anesthesia, MRI, and EEG monitoring. There will be no randomization or treatment assignment as there is no therapy being studied. For successful completion of this protocol, subject

remuneration will be $400. If the study subject is unable to complete the entire protocol, pro-rating of this remuneration will be as follows:

1. Study subjects who complete the medical evaluation but do not begin the anesthesia portion of the study will receive no remuneration.
2. Study subjects who complete the medical evaluation but do not qualify for the study due to medical or safety concerns will receive $25.
3. Study subjects who complete the medical evaluation, and all parts of the anesthesia study, but not the MRI portion, will receive $50.
4. If the study must be stopped due to concerns for the study subject's medical safety, $400 will be given.

Transportation and parking costs will be reimbursed up to $20.

Some study subjects will have the option of participating as a Backup Study Subject for a given session. These study subjects will receive $100 for showing up on the morning of the study at the time designated by the study staff and being available until 10 am to participate in case the primary study subject is unable to participate. That Backup Study Subject will fulfill all the requirements of the primary study subject, including showing up on time, not eating or drinking for 8 hours before the study begins, not taking any sedative drugs for 48 hours prior to when the study begins, and having a responsible adult available to escort you home when you have completed the study. If the Backup Study Subject is called upon and agrees to participate in place of the primary study subject, he or she will receive payment as a primary study subject as detailed above, in addition to the $100 for serving as the backup study subject. Subjects may participate as the Backup Study Subject only once to avoid possible feelings of coersion.

STUDY PROCEDURES

Patient Preparation (for study subjects who will receive propofol anesthesia). Study subjects who will receive propofol anesthesia will arrive at the MGH GCRC at Charlestown. 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.

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 and plethysmography (using chest and abdominal belts) will also be measured. Baseline vital signs will then be taken. A peripheral intravenous line will be placed under local anesthesia (16 - 20 gauge IV, lidocaine 1% intradermal and subcutaneous). An infusion of ringer's lactate will be started. Using a clean technique, an arterial line will be placed in a radial artery under local anesthesia (20 gauge cannula, lidocaine 1% intradermal and subcutaneous). The arterial line will be used for propofol drug sampling. The venous line will be used for administration of propofol. An EEG montage with a maximum of 128 channels will be placed on the subject. Baseline physiologic measurements will be taken.

Airway Management. Induction of general anesthesia will cause predictable changes in respiratory function. General anesthetic agents, including propofol, can produce apnea after loss of consciousness (Taylor et al., 1986). One 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. 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* loss of consciousness. With progressive loss of consciousness, the study subject will hypoventilate and become apneic. Standard airway maneuvers will be used by the attending anesthesiologist 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. With apnea resulting from the increasing plasma propofol concentration, oxygenation and 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%. The subject will be manually ventilated by the clinical anesthesiologist who is present solely for the purpose of medical management (see MONITORING AND QUALITY ASSURANCE). 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.

Study Protocol. The study protocol will be divided into 3 segments. The number of minutes each segment is expected to last is indicated in parentheses. These segments are defined in order as: Baseline Recording (14); Induction and Recovery from Hypnosis (126); Post-Anesthesia Recovery (120).

Baseline Recording Stage. The baseline EEG measurements will be taken as the subject rests quietly for 14 minutes. 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 taken to the post-anesthesia recovery area.

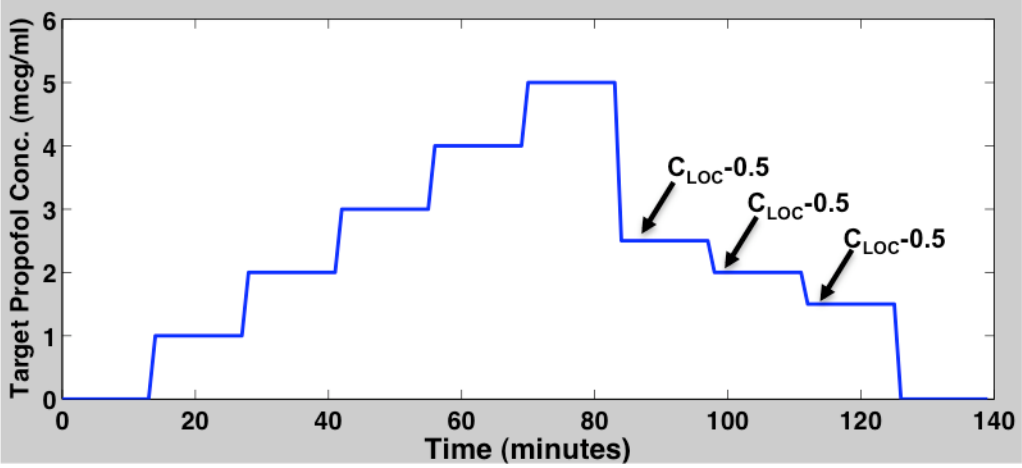
1. EEG acquisition**:** Continuous EEG will be recorded with up to 128 channels. Bandpass filtering of the acquired signal will vary depending upon the features of the EEG data of interest in each experiment. This will typically be from 0.01 Hz to 50 Hz. Trigger signals will be sent from the stimulus 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.

2. Inter-modality coordinate system co-registration: The structural MRI will be registered with the locations of the EEG sensors using a Polhemus FASTRAK digitizing system. Using this digitizing system fiducial landmarks (periauricular points, nasion), EEG electrode locations, and the head outline are digitized in 3D space, and then registered

with the study subject's structural MRI in order to construct an anatomically accurate EEG forward model ((Hari and Lounasmaa, 1989).

3. Video recording to assess level of arousal: Video of the study subject will be recorded during the baseline and induction and recovery from hypnosis periods to assess the study subject's level of arousal after loss of consciousness. In particular, from clinical experience and previous studies, we note that subjects will exhibit varying levels of arousal after 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. Permission to show the video for educational purposes or at scientific presentations will be obtained separately using the standard MGH "Procedure Consent Form," which has a section covering video and photo use. Permission to show the video is entirely optional and not required for participation in the study.

Induction of Hypnosis and Recovery from Hypnosis. Once the baseline measurements are completed, we use a modified version of the paradigm by Kearse et al. to establish loss of consciousness (Kearse, Jr. et al., 1998). We will deliver propofol by infusion using the pharmacokinetic and pharmacodynamic models of (Schnider et al., 1999;Schnider et al., 1998). Propofol will be infused using a previously validated computer-controlled delivery system running the program STANPUMP (Shafer et al., 1988). We will define loss of consciousness (LOC) as the loss of response (button press) to stimulus, and C_{LOC} as the target plasma propofol concentration at which LOC is first observed. We will target, in order, a set of 9 plasma concentrations: 0.0 mcg/mL (baseline), 1.0 mcg/mL, 2.0 mcg/mL, 3.0 mcg/mL, 4.0 mcg/mL, 5.0 mcg/mL, C_{LOC} -0. 5 mcg/mL, C_{LOC} -1.0 mcg/mL, and C_{LOC} -1.5 mcg/mL. If LOC does not occur at a targeted plasma concentration of 5.0 mcg/mL, then the infusion will be stopped and data will be collected as the patient recovers. At each target level, we will wait until the plasma concentration predicted by STANPUMP reaches the target level (usually < ~30 seconds from simulation studies), then wait four plasma effect-site equilibration half-lives (as defined by Schnider – approximately 6 minutes), and define a steady-state period of 7.5 minutes after this equilibration period. Thus, each target level will last approximately 14 minutes. We will take EEG recordings throughout the induction and recovery. Blood sampling for propofol concentration will take place at the baseline level, at the beginning and end of each predicted steady state period, and at the beginning and end of the last 14 minutes of the protocol immediately following the 112 minutes of propofol infusion. Thus, 19 samples will be taken. The total amount of blood sampled will be (19 samples)(15 mL of blood/sample) = 285 mL, 9.6 oz. or 19.2 Tablespoons. Spontaneous recovery from propofol anesthesia will then take place. EEG will be recorded during the first 14 minutes of spontaneous recovery. While the total estimated time is 140 minutes, the study subject will be under propofol infusion for approximately 112 minutes.



At each target plasma concentration, stimuli will be presented depending on the specific aim being studied:

Aim 1 (Auditory): The auditory stimulus set will consist of two components: A) Trains of clicks or noise bursts used to elicit AEPs and B) An intermittent discrimination task to assess the subject's clinical state of consciousness. The clicks or noise bursts will be presented at a rate ranging from 5 to 90 Hz (Harms and Melcher, 2002), 60 dB above hearing threshold, but less than 85 dB SPL. The discrimination task will consist of words, names, and/or natural sounds, contrasted against a "scrambled" version of these stimuli that is perceptually different, but with identical spectral content. The subject will be asked to respond via button-press to identify the target vs. scrambled sounds. The response will be graded (2 if correct, 1 if incorrect, and 0 for no response) and used to assess the subject's level of consciousness. The sound intensity for all stimuli will be set at approximately 60 dB above the subject's hearing threshold, with a maximum of 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).

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Aim 2 (Somatosensory): The somatosensory stimulus set will also use a tone-discrimination task to assess the subject's clinical state of consciousness. The somatosensory stimuli will be provided by a piezo-electric device vibrating at approximately 100 Hz, alternating between vibration and quiescence every 0.5 sec, over a 30 second period. The vibrotactile stimulus will be applied to the subject's non-dominant hand. The tone-discrimination task will be identical to that in **Aim 1**.

Aim 3 (Memory): The memory paradigm will also consist of two components: A) A semantic priming task and B) A tone-discrimination task to assess the subject's clinical state of consciousness. The semantic priming task will be similar to those used in studies by Buckner and Wagner (Buckner et al., 2000;Wagner et al., 2000). The subject will be presented with novel/ repeat trials of an auditory abstract/ concrete semantic discrimination task. Each novel block will consist of 20 words, 10 abstract and 10 concrete, presented at an interval of 2 seconds, followed by a 40-second period of silence. The repeat block following each novel block will be identical to the preceding novel

block except that the words will be in a different order to remove any potential bias due to word ordering. In many ERP priming studies, the subject is instructed to give an "overt" physical response, such as a button press, to indicate the outcome of the task (e.g., button 1 is pressed for "abstract" words and button 2 is pressed for "concrete" words). Recent studies by Buckner have demonstrated that semantic priming differences are seen with both overt and covert responses (Buckner et al., 2000). In this study, the subject will be instructed to perform the task "covertly" or "in their head." In other words, the task will be completed without an "overt" or physically manifested response such as a button press. The tone-discrimination task from **Aim 1** will be presented intermittently in order to gauge clinical level of consciousness.

If the subject becomes apneic with loss of consciousness, he/ she will be manually or mechanically ventilated by the clinical anesthesiologist (see **MONITORING AND QUALITY ASSURANCE**) present solely for the purpose of medical management. The end-tidal (capnogram) carbon dioxide levels will be maintained within 10% of the baseline values. Manual or mechanically assisted ventilation will continue until the subject recovers spontaneous ventilation.

Blood Pressure Management. Decreases in systemic arterial blood pressure are a known, expected side effect of propofol. This is because propofol 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 propofol administration 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 secondary to propofol is advantageous because this counters the propofol-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 (10 mg/ml) will be diluted in 250 ml D5W solution (final concentration 40 ug/ml). The 251 ml bag will be labeled using a standard, yellow phenylephrine identification sticker confirming the contents, concentration and time and date of preparation of the phenylephrine mixture according to usual Department practices. A pediatric buretrol (150 ml) will be placed between the bag and 60 drop/ml microdrip administration set so that the volume administered per unit time can be measured. The distal end of the giving set will be labeled and connected to the distal port of the standard intravenous set already in place. The intravenous phenylephrine infusion will be titrated 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).

Post-Anesthesia Recovery. Following *Induction and Recovery*, the subject will be monitored in a post-anesthesia recovery area for 1 to 2 hours as is done for patients following ambulatory surgery. The subject will be discharged from the post-anesthesia recovery area based on established MGH Department of Anesthesia practices for discharge from the hospital following ambulatory surgery. The subject must have stable vitals 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 and arterial line insertion sites. 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.

Patient Preparation (for study subjects who will not receive propofol anesthesia). Control study subjects will arrive at the MGH GCRC at Charlestown. An EEG montage with a maximum of 128 channels will be placed on the subject. Auditory, somatosensory, or memory stimuli, EEG acquisition, inter-modality coordinate co-registration, and anatomic MRI scans will be identical to those provided to study subjects receiving propofol. In addition, physiological measurements will be made, consisting of non-invasive blood pressure, ECG, pulse oximetry, and galvanic skin response. EEG recording will last approximately 1 hour. Video will not be taken for non-anesthesia study subjects.

Anatomic MRI Scan.
Structural MRI will be performed using a standard head coil on a 1.5T or 3T scanner. These high resolution anatomic scans will be used to construct a realistic head model for each study subject for use in the forward model. The structural MRI acquisition will be performed on a separate day from the EEG acquisition. The study subject will be able to verbally communicate with the investigators during the structural MRI portion.

Statistical Data Analysis
All data will be stored on CD-ROM for subsequent off-line analysis. Study subject blood samples will also be forwarded to an external laboratory for propofol assay determination. All blood samples will be coded and de-identified prior to forwarding for assay. The spectral analyses, bispectral analyses, AEP's estimates, and source localization estimates will be computed from the raw EEG signal in off-line analysis. For source localization analysis, a 3-layer boundary element forward model will be constructed from the anatomic MRI scans (Liu et al., 2002;Oostendorp and van Oosterom, 1989). Inverse calculations will be performed using the minimum-norm estimate (Hamalainen et al., 1993). In all the analyses, the EEG signal processing estimates will be correlated with changes in behavior on the specific task and the target propofol plasma concentrations.

Anticipated Findings. EEG source localization will be co-registered with anatomic MRI scans to follow changes in neuronal activity during induction and recovery from propofol anesthesia. Changes in EEG recordings to auditory, somatosensory, and semantic repetition priming stimuli will provide insight into how anesthesia modulates auditory

processing, loss of sensation and loss of memory. We anticipate that each **Aim** will take approximately 20 weeks to complete, if we are able to impanel one subject per week.

RISK AND DISCOMFORT

Anesthetic Risks

The anesthetic risks are burning on injection of the propofol, allergic reactions or seizures from lidocaine local anesthetics, hypertension or bradycardia secondary to phenylephrine, apnea, loss of airway control, hoarseness, nausea and vomiting, discomfort from the arterial line placement, and discomfort from the intravenous line placement. The most significant risk to the subject is anesthetic induced apnea. One 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. In addition to the anesthesiologist responsible for the medical management of the patient, there will be a team of two other advanced cardiac life-support certified professionals available for the duration of the study to assist in the event of a medical emergency. The team will consist of either 2 anesthesiologists, or one anesthesiologist and another physician or nurse.

EEG Risks

EEG electrodes placed on the scalp may cause temporary redness.

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).

Psychological Risks

Psychological risks include the possibility of claustrophobia within the scanner.

POTENTIAL BENEFITS

There are no direct benefits to the individual subjects involved in this study. The potential benefits of this study to society are co-registration of EEG source localization with anatomic MRI scans to follow changes in neuronal activity during induction and recovery from propofol anesthesia. Furthermore, changes in EEG recordings to auditory, somatosensory, and semantic repetition priming stimuli will provide insight into how anesthesia modulates auditory processing, loss of sensation and loss of memory.

MONITORING AND QUALITY ASSURANCE

All MRI images, EEG and bispectral data will be stored for later off-line analysis. Safety monitoring will include the presence of two anesthesiologists for the conduct of the study, immediately available cardiopulmonary resuscitation cart, oxygen, ambu bag and defibrillator for use in need of an emergency. A fully functional 0_2 delivery system, monitors and a fully stocked anesthetic cart with equipment for airway management will also be available. One anesthesiologist, the clinical anesthesiologist, who is not taking

part in the execution of the study protocol will be present at all times and will be 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. In addition to the anesthesiologist responsible for the medical management of the patient, there will be a team of two other advanced cardiac life-support certified professionals available for the duration of the study to assist in the event of a medical emergency. The team will consist of either 2 anesthesiologists, or one anesthesiologist and another physician or nurse. A standard checkout protocol for the oxygen delivery system, anesthesia cart, and the resuscitation cart will be carried out prior to the start of each study. In the event of an emergency, each member of the study team will have a specific task to execute. The emergency protocol will be reviewed prior to the start of each study (see attached "Emergency Protocol" sheet).

All outcome monitoring and adverse events will be reported through appropriate channels of the Human Studies Committee.

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