Study Title: Magnetic Resonance Imaging of the Effect of Music Listening on Brain Activity Under Anesthesia

NCT: NCT04464265

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IRB Document Approval Date: 4/1/2021

MR imaging of the Effect of Music Listening on Brain Activity under Anesthesia

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General Goal

The goal of this study is to better understand the systems-level neuronal mechanisms by which general anesthetics produce loss of consciousness under our NIH-funded project (R01-GM103894). Our general hypothesis has been that anesthetics suppress consciousness by disrupting the functioning of large-scale brain networks that support information integration in the brain. We will study brain processes involved in rich and naturalistic situations using complex, temporally structured stimuli (i.e. music). We will determine the neural correlates of distorted perception under sedation by using blood-oxygen level-dependent (BOLD) functional MRI (fMRI). This study should yield insight into how sedation may alter the temporal integration of complex stimuli along the brain's functional hierarchy that supports conscious experience. Taken together, the study should advance our understanding of the fundamental neuronal mechanisms of anesthetic modulation of conscious cognition with a significant translational impact for the clinical assessment of the state of consciousness.

Rationale and Hypothesis

Real-world events are processed via hierarchically organized temporal receptive windows (TRWs) in the brain (*1*–*5*). The TRW of a neuron is defined as the length of time before a response during which sensory information may affect that response (*1*). Hence, the TRWs reflect the timescale on which specific brain region typically processes information. Namely, short TRW of early sensory areas process rapid sensory information, whereas longer TRW of higher order brain areas integrate perceptual and cognitive events that unfold over longer time periods (*6*). Therefore, the lengths of TRWs across brain regions can delineate the brain's spatially distributed functional hierarchy. How sedation alters the brain's functional hierarchy accounting for the distorted perception of complex, temporally extended stimuli is an important scientific question.

We hypothesize that sedation breaks down the functional hierarchy by prolonging the brain's intrinsic timescales (increase the TRWs; Fig. 1). This disrupts the functioning of large-scale brain networks that support perceptual integration. To test this hypothesis, we will measure the TRWs from resting-state (intrinsic) and music listening (extrinsic) periods during both conscious wakefulness and sedation. We anticipate observing a global prolongation of intrinsic TRWs, where the enlarged TRWs act as a low-pass filter of extrinsic sensory inputs, and cause the failure of high-order information integration over longer timescales (e.g. in prefrontal regions) during sedation.

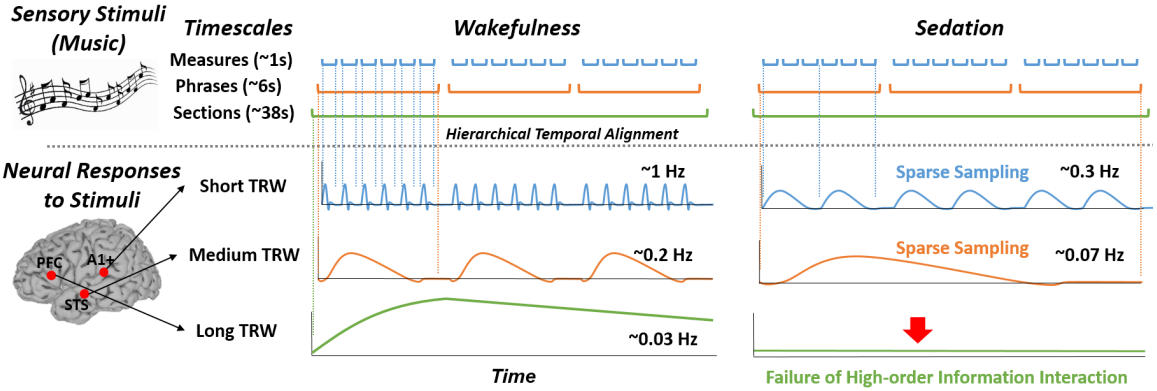

Fig. 1. Hierarchical organization of temporal receptive windows (TRWs) and their measurement during wakefulness and sedation. During wakefulness, music is processed via hierarchical TRWs in the brain, where the timescales of neural responses (frequencies in Hz) to stimuli are matched with the multiple timescales of sensory stimuli. During sedation, the TRWs are enlarged such that they act as a low-pass filter or sparse sampling of the sensory inputs. The mismatch between the brain's TRWs and the timescales of sensory inputs may not only affect information acquisition at relatively short timescales but may also fail high-order information integration over longer timescales in, for instance, prefrontal regions with long TRWs.

Number of Subjects, Recruitment and Informed Consent

Statistical power analysis (see below) requires a group of 25 subjects. We plan to recruit a total of 30 subjects (ages between 18-40 years old) to complete the study considering the possible failure or attrition. Healthy participants will be recruited by listing on UMClinicalStudies.org and by postings at area colleges and community groups in Ann Arbor. Interested volunteers will call the phone number of a designated recruiter for an initial phone screening. The initial phone screening will consist of questionnaires related to medical history, demographic information, handedness, inclusion and exclusion criteria and procedure standard MRI screening questionnaire. If interested, the participant will complete the questionnaires, which will be reviewed by the study team. The health status will be confirmed by the attending anesthesiologist before the study on site. Once eligibility is confirmed by the study team, the one-time research study session will be scheduled. All eligible participants will be required to take a COVID-19 nasal swab test within 48 hours of their scheduled study visit date, consistent with the Michigan Medicine Operating Room Covid Testing Policy. The study team will place the order and will provide a list of MLAB locations where this test can be performed. This screening test will be paid for by the study. A negative COVID-19 test must be resulted prior to their scheduled study visit date. Participants will again be screened for COVID-19 prior to entry into the hospital per hospital guidelines. A standard face mask will be provided upon hospital entry if participants do not have one.

All participants will give written informed consent according to institutional guidelines prior to any testing. The Principal Investigators or their designee will obtain consent using a written consent form approved by the Institutional Review Boards of the University of Michigan Medical School (IRBMED). It will contain detailed information regarding the purpose, risks and benefits of participating. Copies of the signed consent form will be given to the subjects; the original consent will remain with the study team. Subjects will be compensated for their involvement.

Inclusion and Exclusion Criteria

Volunteers are screened using a medical history and demographics questionnaire.

Inclusion Criteria. The subject population will consist of healthy study subjects with ASA-1 status. The participants' health status will be assessed by the attending anesthesiologist prior to inclusion in the study. The participants will be right-handed adults between the ages of 18 and 40 with a body mass index (BMI) less than 30. All subjects will be English speakers.

Exclusion Criteria. Participants will be excluded if they have any medical contraindication to MRI scanning; are unable to undergo MRI scanning because of possible pregnancy or currently breastfeeding, BMI>30, metallic substances in the body, claustrophobia, anxiety, or cardiopulmonary disease; or have an intracranial structural abnormality on T1-weighted MRI scans. Potential subjects will be excluded if they have a history of allergy to propofol, eggs or egg products, soybean or soybean products, neurological, cardiovascular, or pulmonary illness; significant head injury with loss of consciousness; learning disability or other developmental disorder; sleep apnea or any severe snoring history; gastroesophageal reflux disease (GERD) or heartburn; pancreatitis or a history of pancreatitis, or sensory/motor loss sufficient to interfere with performance of the study. Participants with tattoos in the head or neck region will be excluded from study; other tattoos are subject to determination by investigators based on their assessment regarding participant safety. To eliminate aspiration risk subjects will also be excluded if they have

had recent food or liquid intake (within 8 hours). Subjects will be excluded if they have a history of drug use, have a positive drug screen, are unwilling to abstain from alcohol for 24 hours prior to dosing, or have a current history of nicotine use. Women will be required to take a pregnancy test prior to participation to ensure a negative result. The pre-scan drug screen and pregnancy test will be paid for by the study.

Procedures

Imaging. Noninvasive functional magnetic resonance imaging will be performed in the Philips 3T (MR2) Research MRI (standard 32-channel transmit/receive head coil) at the University of Michigan Health System, University Hospital, Department of Radiology.

1) SPGR high-resolution images. T1 weighted spoiled gradient recalled echo (SPGR) images will be acquired for high spatial resolution of anatomical images with parameters: 170 sagittal slices, 1.0mm thickness (no gap), TR=8.1s, TE=3.7ms, flip angle=8^{o}, FOV=24cm, image matrix 256×256.

2) Functional MRI images. A gradient-echo EPI pulse sequence will be employed to acquire functional images over the whole brain with parameters: 28 slices, TR/TE=800/25ms (multiband fMRI acquisition), slice thickness=4mm, in-plane resolution=3.4×3.4mm; MB factor=4; field of view (FOV)=220mm, flip angle=76°, image matrix: 64×64. Verbal instructions were presented through earphones. Eight fMRI scans (16-min per scan) will be conducted throughout the experiment. The eight scans will include 16-min resting-state and 16-min music-listening during wakefulness baseline; 16-min behavioral test during propofol induction; 16-min resting-state and 16-min music-listening after loss of behavioral responsiveness; 16-min behavioral test during emergence period (after propofol infusion is terminated); 16-min resting-state and 16-min music-listening after recovery of behavioral responsiveness. There will be a 1-5 minute break in between scans. The imaging protocols and data acquisition will be completed within 2.5 hours scanning session for each subject.

Anesthesia. Sedation will be achieved by target-controlled IV infusion of propofol (see **Appendix I** below for detailed plan). The IV line will be placed after application of a local anesthetic. Propofol will be administered by intravenous infusion. Nasal cannula will be used for supplemental O2 and CO2 sampling. All anesthesia equipment, supplies, and drugs will be provided by anesthesiologists from the University of Michigan Health System. We will manually control the infusion of propofol to achieve target effect-site concentrations of 1.5, 2.0, 2.5, and 3.0 μg/ml in a stepwise fashion. Each target concentrations will be maintained for 4 minutes. In this way, we will be able to titrate the anesthetic level using those dose increments to reach the point of loss of behavioral responsiveness (LOR). In order to minimize head motion-related artifacts, the effect-site concentration will be maintained at one step above the expected concentration for LOR for approximately 32 minutes. For example, if a participant loses responsiveness at 2.5 μg/ml, we will maintain 3.0 μg/ml effect-site concentration during the entire LOR period (Fig. 2). For exceptional cases when participants retain responsiveness at 3.0 μg/ml (occurrence rate of ~5.6% in our previous studies), we will increase target effect-site concentrations to 3.5 μg/ml (assuring LOR in 99.5% population) and maintain at maximum of 4.0 μg/ml. After that, the infusion will be terminated and the propofol concentration is allowed to gradually decrease. Participants will be instructed to perform a behavioral test, taking a rest, or listen to the music as described below.

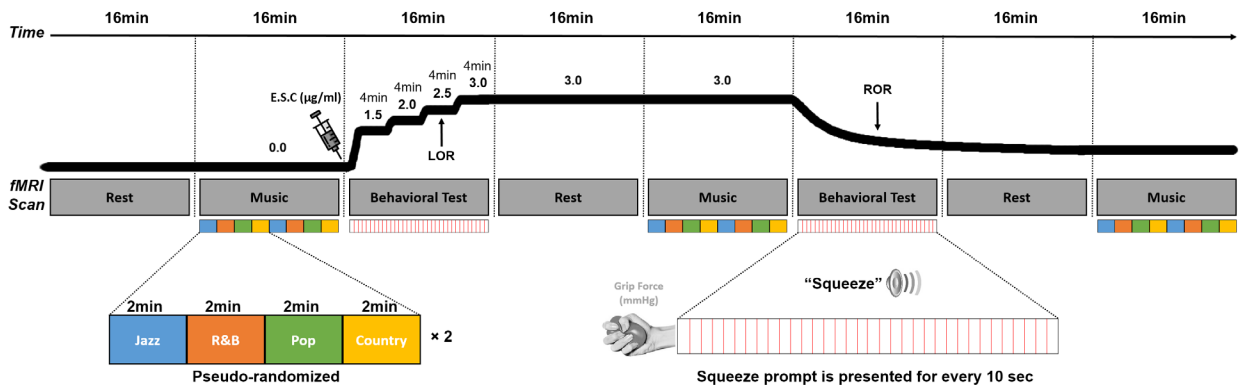

Fig. 2. Experimental design for fMRI. Figure shows hypothetical case in which the participant loses responsiveness (LOR) at propofol effect-site concentration (ESC) of 2.5 μg/ml.

fMRI Protocol. During the rest-state period, participants will be asked to lay at rest with eyes closed, while trying to stay awake without thinking of anything in particular, and trying not to move. During the music presentation period, participants will be asked to listen to the music, keeping their eyes closed, try not to move, and stay awake. Four types of music, including Jazz, Rock, Pop and Country, will be presented in a pseudo-randomized order. We will select well-known music excerpt and being evaluated by all participants using familiarity and meaningful scales before the experiment. Each piece of music will be edited into 2-min duration. During the behavioral test period, participants will be asked to squeeze an MRI compatible grip dynamometer (a rubber ball) for every 10-second periods (96 cycles in total). The beginning of each cycle will be cued with the spoken word "squeeze".

The verbal instructions will be programmed using E-Prime 3.0 (Psychology Software Tools, Pittsburgh, PA) and delivered via an audiovisual stimulus presentation system designed for an MRI environment. The volume of the headphones will be adjusted for subject comfort during wakefulness. The volume will be increased to 150% after loss of responsiveness. Behavioral responses will be measured in mmHg of air pressure during squeezing the rubber ball, using BIOPAC (https://www.biopac.com) MP160 system with AcqKnowledge software (V5.0). By comparing the timing of "squeeze" instructions (expected motor response) and the actual motor response during and after stepwise propofol infusion, the periods during which a subject loses of responsiveness (LOR), and recovery of responsiveness (ROR) will be determined. The onsets of LOR and ROR were defined at the time of first failure to squeeze, and the first success to squeeze, respectively.

All testing will be performed by the attending anesthesiologists. Spontaneous respiration, end-tidal CO_2, heart rate, and electrocardiogram will be continuously monitored. Noninvasive arterial pressure will be measured with magnetic resonance (MR)-compatible automatic monitor (BIOPAC). Post anesthesia care will consist of checking vital signs for 1 hour by the nursing staff. A wheelchair will be provided to assist the participant if needed. An anesthesiologist will be present during the entire duration of the study.

Primary outcome measure: Blood Oxygen Level Dependent (BOLD) Response to sensory stimuli during sedation (timeframe: baseline to 90 minutes).

Secondary outcome measure: Squeeze Pressure, defined as measurement of force of participants' hand squeezing on a rubber ball in response to instructions (timeframe: baseline to 90 minutes).

Data Analysis

Image analysis will be conducted using AFNI (http://afni.nimh.nih.gov/afni). 1) The first two frames of each fMRI run will be discarded; 2) Slice timing correction; 3) Rigid head motion correction/realignment within and across runs; frame-wise displacement (FD) of head motion will be calculated using frame-wise Euclidean Norm (square root of the sum squares) of the six-dimension motion derivatives. A frame and its each previous frame will be tagged as zeros (ones, otherwise) if the given frame's derivative value has a Euclidean Norm above FD=0.5 mm; 4) Coregistration with high-resolution anatomical images; 5) Spatial normalization into Talaraich stereotactic space; 6) Using AFNI's function 3dTproject, the time-censored data will be high-pass filtered above 0.008 Hz. At the same time, various undesired components (e.g., physiological estimates, motion parameters) will be removed via linear regression. The undesired components include linear and nonlinear drift, time series of head motion and its temporal derivative, binarized FD time series (output data included zero values at censored time points), and mean time series from the white matter and cerebrospinal fluid; 7) Spatial smoothing with 6 mm full-width at half-maximum isotropic Gaussian kernel; 8) The time-course per voxel of each run will be normalized to zero mean and unit variance, accounting for differences in variance of non-neural origin (e.g., distance from head coil).

The music evoked activity, will be quantified by inter-subject correlation (inter-SC) of the fMRI responses (*7*), depends systematically on the music structure coherence. For instance, early auditory areas respond reliably to low-level acoustic features of music, whereas frontal areas process long-timescale musical structures, on the order of minutes. This analysis provides a measure of the reliability of the responses through the functional hierarchy by comparing the BOLD response time courses across different subjects. The inter-SC analysis will be performed on a voxel-by-voxel basis. First, the Pearson product-moment correlation will be computed between a voxel's fMRI time course in one individual and the average of that voxel's fMRI time courses in the remaining subjects. Next, the average correlation coefficient will be calculated at every voxel. Finally, it will produce an average inter-SC map during music presentation period. Statistical significance of inter-SC for each state (i.e. wakefulness baseline, LOR period, and ROR period) and the difference between states (e.g. wakefulness baseline vs. LOR period) will be assessed using a phase-randomization procedure (forming a null distribution).

Intrinsic TRWs will be calculated from the resting-state data. We will use Mean Frequency (MF) of fMRI signal fluctuations to index the TRW length (*8*). Specifically, power spectrum will be obtained by computing the periodogram of each voxel time series using AFNI program 3dPeriodogram. Tapering was applied using the Hamming window to reduce bias and error variance for spectral estimation in finite data (*9*). The MF of a spectrum will be calculated as the sum of the product of the spectrogram power intensity and the frequency, divided by the total sum of spectrogram power intensity:

$$MF = \frac{\sum_{i=0}^{n} P_i F_i}{\sum_{i=0}^{n} P_i}$$

where n is the number of frequency bins in the spectrum, Fi is the frequency of spectrum at bin i of n, and Pi is the power intensity of spectrum at bin i of n. Lower MF indicates a shift toward a longer timescale or slower dynamics.

Anticipated Results and Implications

We expect to find a spatial hierarchy with reliable responses (inter-SC) to music starting in the primary auditory cortex and adjacent areas of auditory cortex (A1+), processing along the superior temporal sulcus (STS) and further up to the middle and inferior frontal gyrus during conscious wakefulness. We anticipate that during LOR, inter-SC in especially the frontal areas, which process long-timescale musical structures, will be reduced. We also expect to observe that, during

LOR, the intrinsic timescales derived from the resting-state are overall prolonged, and the intrinsic functional gradient of hierarchy is also shrunk. The degree of the intrinsic TRW prolongation and the degree of the functional gradient shrinkage may show a negative correlation with the spatial extension of inter-SC during sedation.

Together, the results should advance our understanding of how the brain processes information at increasing levels of abstraction along the functional hierarchy, and how this information processing may be altered during sedation. The information gained should add further insight into the neural mechanisms for temporal integration in the brain supporting conscious experience of complex stimuli and their degradation under sedation.

Power Analysis

For an fMRI study of cognitive function, Desmond and Glover (*10*) reported that about 25 subjects are necessary to achieve 80% power for a 0.5% increase of activity. We also conducted power analysis based on our previous study with graded sedation of propofol (*11*). The average Cohen's d as a measure of the effect size was 0.56. For 80% power and alpha=0.05, N=24 human subjects will be needed. The obtained subject number is also very close to the suggested optimal group size for reliable statistics in functional MRI studies (*12*). To account for possible failure or attrition, we plan for 30 as the maximum number of subjects.

Biological Variables

We will recruit participants of both sexes with various ethnic/racial backgrounds, aiming for diversity and equal representation. The age range will be limited to 18-40 years in order to limit age-dependent variation in anesthetic requirement for sedation and for participant safety.

Potential Risks

There are no known risks associated with magnetic resonance imaging, as long as technical parameters remain within FDA guidelines. A licensed MR technologist is instructed in these guidelines and performs all machine manipulations. Complete histories, physical examinations and stringent medical guidelines exclude many potential subjects before they enter the study, thereby further minimizing risks. A crash cart is always present and regular safety drills are performed by the medical and experimental staff to prepare for any untoward effects.

Risks related to MRI scanning:

MRI Static Magnetic Fields: FDA guidelines for clinical product scanners limit the main magnetic field to 3 Tesla. Our studies will be performed on a standard, clinical 3 T Philips scanner. A substantial body of literature exits that supports the safety of field strengths up to 7 T. There are no known risks of exposure to 3 or 7 T fields for MR imaging.

Among the reported biological effects of exposure to strong static magnetic fields are the following: 1) Dizziness (with nausea) or stimulation of the sensory nerves in the soft palate has been reported as a result of the subject's head being moved while in the magnet. 2) Rapid eye movement has been reported (at 4 and 7 T) to cause magnetophosphene effects. First described in 1896, these flickering light sensations are similar to visual phenomena caused by direct electric current passage across the head or retina.

Radiofrequency (rf) Magnetic Fields: Radiofrequency energy burns are the result of high electric fluxes in the immediate vicinity of the patient. All MRI systems create some rf electric fields incidental to the production of the intended rf magnetic fields. The potential causes are malfunctions in the rf coil switching circuitry, improper use of the surface coils, design flaws in some clinical MRI system surface coils or cardiac gating patient connection leads. Nationally, 60 burn incidents have been reported to the FDA in the course of approximately 11 million exams. Our site has never experienced any difficulty in this regard.

Acoustic Noise: The sound generated by an MR system usually consists of a series of repetitive pulses. The relevant safety parameters required to characterize such a noise are the peak impulse sound pressure level (L_{peak}) and the time integral of the A-weighted sound pressure level (L_{eq}). In MR applications, the peak impulse sound pressure level is dependent upon the peak amplitude of the individual pulses, while the time integral of the A-weighted sound pressure level is dependent upon the continuous exposure to a series of such pulses. Acoustic noise is a result of the mechanical vibration produced by the gradient coils when the nominally large currents are applied to them to create time varying imaging gradient fields. The sound produced can be loud enough to produce temporary deafness (up to 95 dB). Foam earplugs will attenuate this noise by up to 30 dB. They are in routine use at the 3T systems, and have been proven effective.

Claustrophobia: While inside the magnet, subjects may experience an acute panic attack due to claustrophobia. Subjects are all prescreened for fear of tight places. Once inside the magnet, the subject will be given a squeeze ball to signal the MR operator if he or she is under acute distress.

Risks related to anesthesia:

Risks associated with intravenous catheter placement include brief, local discomfort and possible bruising, which can occur at the site of the IV line. There is also a possible (rare) chance of infection, but every precaution will be taken to reduce this risk by keeping the IV site clean and dry.

Risks associated with propofol administration include: infection due to microbial contamination, pain at the site of injection, possible allergic reaction to the drug, respiration depression which may necessitate placement of an endotracheal tube to assist breathing, and mechanical ventilation, or a decrease in blood pressure which may necessitate giving a drug or IV fluids to bring the subject's blood pressure up, and hyperlipidemia. These effects will be minimized by employing individualized dosing utilizing the STANPUMP software (Shafer, 1996), avoiding large bolus doses or rapid increases in drug administration, and careful clinical monitoring during drug administration. These effects disappear when propofol is discontinued.

Justification for the risks involved

Although the risk of this study is minor increase over minimal, the importance to mankind of the potential knowledge gains is substantial, making the risk/benefit ratio very low. We combine the state-of-the-art brain imaging facility currently in place in the University of Michigan Health System with the experience of the imaging staff and clinic anesthesiologists in the Department of Anesthesiology. These experiments will provide a unique opportunity to understand the neurophysiological mechanisms of anesthesia modulation of human consciousness. Therefore, we will provide a unique opportunity to expand our knowledge of anesthesia-modulated human consciousness.

Procedures for minimizing risks

All research subjects will go through a screening process for inclusion and exclusion criteria performed by licensed physician. A licensed physician will be present at all MRI scanning sessions to monitor the subjects. When subjects express or exhibit anxiety regarding the scanner setting, they will be allowed to discuss these feelings. Attempts will be made to minimize the discomfort and anxiety. When in the scanner, subjects will be provided with a squeeze-ball alarm that will alert the scanner technician and the researcher that the subject desires to come out of the scanner. In such instances, the subject will immediately be removed from the scanner and appropriate measures will be taken to ensure his or her psychological health. Earplugs, demonstrated to reduce scanner noise to a non-damaging level, will be used to minimize hearing risks.

In addition, any other health problem that would be aggravated by an MRI scan will also be a cause for terminating the subject's participation. In the event of serious cardiac-respiratory problems or other medical emergencies, the hospital code team will be activated. Other medical problems will be treated by the monitoring physician. Any medical problems that arise after release, although unlikely, will be treated by a physician from the research team. All subjects will have to arrange transportation after the study, and agree to not drive, work, operate machinery, or make legal decisions for 24 hours after the study. Subjects will be informed of their right to withdraw from the study at any time.

A protocol has been established to handle unexpected medical emergencies arising in the scanner suite. This protocol deals with medical issues and the duties of each team member. This includes stopping the scanner, removing the patient and calling for outside medical assistance. All members of the research team participate in periodic emergency protocol drills in the scanner suite and a copy of the written protocol and protocol termination guidelines are present at all times. Subjects requiring intervention for any neurological or cardiovascular adverse event will be transferred to the Emergency Department for further evaluation and treatment as seen fit by the attending physician. More serious cardiac events such as ventricular tachycardia, ventricular fibrillation or any unstable rhythm will be immediately treated according to current Advanced Cardiovascular Life Support (ACLS) protocols. Research team physicians have immediate access to valium, diphenhydramine, epinephrine, lidocaine, and NTG for emergency use, in addition to a fully supplied and operational "crash cart" with EKG, defibrillator, suction, oxygen, and airway management equipment.

Confidentiality

Strict subject confidentiality will be maintained. Subjects will be assigned a code number following their first contact in the protocol. This number will be used throughout the experiment and will be the only identifier on behavioral and physiological archival data, and magnetic resonance (MR) scans. The identity of subjects will not be revealed at scientific meetings, in publications or other vehicles of public communication. Data will be pooled across subjects where appropriate. Only the PIs, Co-investigators, and the study coordinator will have access to the ID code, which will be stored in a locked file separate from the data.

Any behavioral data collected on the subjects will be coded by study ID numbers and entered directly into notebook computers used in the field. These computers are password protected and stored behind a locked door when not in use. Clinical and biographic data are entered into the database using the ID number only. Only select staff members have access to the actual paper copies. Medical information will be released by name only to health care providers, and then only with written permission from the subject.

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Appendix I. Sedation Protocol

Anesthesia. Sedation will be achieved by target-controlled IV bolus+constant rate infusion of propofol (see similar protocol in Huang et al., 2018, 2020). The IV line will be placed after application of a local anesthetic. All anesthesia equipment, supplies, and drugs will be provided by anesthesiologists from the University of Michigan Health System. Propofol is provided as a ready-to-use formulation with a concentration of 10mg/ml. However, should dilution be necessary, it will be diluted with 5% Dextrose Injection, USP, and will not be diluted to a concentration less than 2 mg/mL because it is an emulsion. Vials will not be accessed more than once, or used on more than one subject.

We will manually control the infusion of propofol to achieve target effect-site concentrations of 1.5, 2.0, 2.5, and 3.0 μg/ml in a stepwise fashion (no other drugs will be administered). The target concentrations are based on our previous study (Huang et al., 2018, 2020). The bolus dose, infusion rate and infusion duration for each target concentration and for each participant will be pre-determined based on a pharmacokinetic model (Marsh et al., 1991) developed for target-controlled propofol infusion and implemented in software (STANPUMP; Shafer, 1996). The dosing (bolus+infusion) will be incremented at every 4 minutes until the final target is reached. The incremental dosing will be used to titrate the anesthetic level to the point of loss of behavioral responsiveness (LOR); that is, a clinical end point. In order to minimize head motion-related artifacts, the final effect-site concentration in each participant will be one step above that produces LOR and will be maintained at this level for approximately 32 minutes. For example, if a participant shows LOR at 2.5 μg/ml target concentration, we will use as final target 3.0 μg/ml. Fig. 2 illustrates the propofol dosing for this case, as an example. Note that bolus and infusion rate is given in mcg/kg (ug/kg) and mcg/kg/min (ug/kg/min), respectively.

The table below provides the pre-calculated propofol dosing for a female participant (age, 21yrs; height, 170cm; weight, 70kg).

Target Site Conc. (μg/ml)	Time (min)	Bolus Dose (μg/kg)	Bolus (mg)	Pump Off After Bolus (min)	Time Infusion Start (min)	Infusion Rate (μg/kg/min)
1.2	0	420	29	1:40	1:40	69.7
1.6	4	201	14	1:00	5:00	87.8
2.0	8	213	15	1:00	9:00	105.3
2.4	12	224	16	0:50	12:50	122.0
2.4	16	-	-	-	16:00	104.7
2.4	48	Pump Stop				

Target Site Conc. (µg/ml)	Time (min)	Bolus Dose (µg/kg)	Bolus (mg)	Pump Off After Bolus (min)	Time Infusion Start (min)	Infusion Rate (µg/kg/min)
1.5	0	525	37	1:40	1:40	87.0
2.0	4	265	19	1:00	5:00	110.1
2.5	8	282	20	1:00	9:00	131.7
3.0	12	301	21	0:50	12:50	152.5
3.0	16	-	-	-	16:00	128.2
3.0	48	Pump Stop				

For each effect-site target concentration (Column 1) propofol bolus is given at 4-minute intervals (Time, Column 2) followed by a wait period for equilibration (Pump Off, Column 5) after which constant-rate infusion starts (Time Infusion Start, Column 6) at pre-determined infusion rate (Infusion Rate, Column 7). At the final target (3.0 µg/ml), a second infusion is initiated at 16 minutes (Column 6) at an infusion rate of 128.2 µg/kg/min in order to maintain the effect-site concentration of 3.0 µg/ml. At 48 minutes, the infusion is terminated (Pump Stop) and the propofol concentration is allowed to gradually decrease.

Participants will be instructed to perform a behavioral test, take a rest, or listen to the music as described above.

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