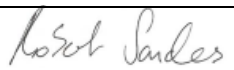
Clinical Trial Research Protocol

Protocol Title: UNderstanding CONSciousness Connectedness and Intraoperative Unresponsiveness Study-3: a randomised, double-blind, cross-over trial in healthy volunteers (UN-ConsCIOUS-3)

Protocol Number	**X23-0174**
Coordinating Principal Investigator	Professor Robert D. Sanders MBBS, Ph.D.*
Signature: 	**Date: 18.05.2023**
Protocol Authors (Co-investigators)	Dr. Tim McCulloch Dr. John Loadsman Miss Kaitlin Kramer
Sponsor (if applicable)	Sydney Local Health District

Ethics Statement:

The study will be conducted in accordance with the National Statement on Ethical Conduct in Human Research (2007), the CPMP/ICH Note for Guidance on Good Clinical Practice and consistent with the principles that have their origin in the Declaration of Helsinki. Compliance with these standards provides assurance that the rights, safety and well-being of trial participants are respected.

SUMMARY

Protocol Title	UNderstanding CONSciousness Connectedness and Intraoperative Unresponsiveness Study-3: a randomised, double-blind, cross-over trial in healthy volunteers (UN-CONSCIOUS-3)
Objectives	The primary objectives of UNCONSCIOUS 3 are: The incidence of conscious state between TACS and sham stimulation under steady state dexmedetomidine. The secondary objectives are: 1. The incidence of disconnected conscious experience (dreaming) versus connected conscious experience (awareness of the external world) between TACS and sham stimulation and no stimulation. 2. The neural correlates of consciousness in anterior and posterior cingulate as identified by source reconstruction of beta/delta EEG power and other EEG analyses. 3. Differences in resting state power, evoked and induced responses between wakefulness, connected consciousness, disconnected consciousness and unconsciousness. 4. EEG responses and time to emergence with 15 minutes of TACS or sham.
Study design	Single-site, randomised, double-blind cross-over trial in healthy volunteers
Planned sample size	20
Selection criteria	Patients recruited will be 18-40 years old without contraindication to anaesthesia or allergy to study drug.
Study Procedure	Telephone screening, eligibility, basic medical history. Sedation visit: Anaesthetic assessment, medication review, vital signs and pregnancy test. Procedural sedation by an anesthetist. Completion of predictive coding and cognitive tasks, auditory stimuli, transcranial alternating current stimulation (TACS), and follow up questionnaire. *none of the procedure in this study are considered standard treatments.
Statistical considerations	The sample size estimate assumes a beta of 0.8 and a two-side alpha of 0.05. Based on prior data, we expect that 60% of the wake ups from dexmedetomidine will be associated with disconnected consciousness during sham stimulation (assuming no difference from 'no sham') and we assume that TACS will reduce the incidence of disconnected consciousness by one third (absolute decrease of 20%, standard deviation of 30%). Based on these assumptions we require a total of 20 patients (assuming 15 wakeups with TACS stimulation and 15 wake ups with sham stimulation) and a 20% loss to follow up.
Time Period of Data Collection	1 year
Duration of the Study	5 years
Sponsor (if applicable)	Sydney Local Health District

Protocol Version Control box

Protocol Version Number	Date	Summary of Changes
1 and 1.1	18.05.2023	Initial iterations
1.2	24.07.2023	Edits made as per Ethics Review- added cross-over trial to design, edits to sections 6, addition of study flow diagram, addition of section 19, edits to section 4.1, added risks of TACS, formatting and edit to appendices.

Contents

1. BACKGROUND AND INTRODUCTION

1.1. DISEASE/PROPOSED INTERVENTION BACKGROUND

'Consciousness' is synonymous with 'subjective experience': it can be summarized as "what abandons us every night when we fall into dreamless sleep and returns the next morning when we wake up or when we dream." Similarly, patients typically report the absence of subjective experience upon recovering from anaesthesia, a state of unconsciousness, though they sometimes also report dreams (consciousness that is disconnected from the environment). As such anaesthetics appear to produce a reversible state of unconsciousness accompanied by amnesia[1]. This remarkable phenomenon brings great relief to surgical patients and wonder to clinicians and scientists. How this is achieved, remains one of the most important scientific questions and will illuminate the fundamental mechanisms of human consciousness and cognitive itself. However, it is important to note that, consciousness also occurs during anaesthesia: dreaming is common under clinical conditions (33% of patients) and in laboratory studies (up to 80% of participants[2]) but participants typically remain "disconnected" from

their environment (i.e.. they may be in a dream-state, unaware of their environment and the external sensory world)[1].

The intense interest in "depth-of-anaesthesia" monitors demonstrates its perceived importance among practicing clinicians. Just as monitors of cardio-respiratory function dramatically improved patient safety, a reliable monitor of consciousness has the potential to reduce the incidence of awareness with recall, which remains unacceptably high (up to 0.2%) and can lead to adverse psychological sequelae, such as symptoms of post-traumatic stress disorder. At the same time, there is a concern that many patients may be "overdosed" in an attempt to minimize awareness with recall. Current approaches to measuring depth-of-anaesthesia suffer from significant inter-patient variability in response properties. They also lack predictive power: although valid on average, they fail too often to report the level of consciousness in individual patients[1]. We suggest that an approach based on a fundamental understanding of consciousness is an essential path to identifying a universally applicable measure that can be used to guide drug administration in the perioperative setting.

Understanding how we may be conscious under anaesthesia but unaware of our environment (sensory disconnection) is also critical[1]. Our ConsCIOUS1 study has shown that 4.6% of patients are aware of their environment following intubation under general anaesthesia[3]. ConsCIOUS2 showed that 11% were conscious and aware of their environment under intended general anaesthesia. Presently we lack markers of consciousness or disconnection that provides confidence that sensory stimuli will not trigger a conscious experience. Such a marker would have clinical utility to identify patients at risk of anaesthesia awareness. As discussed above, dreaming (disconnected consciousness) is common under clinical anaesthesia. A depth of anaesthesia monitor that detected internally triggered dreaming (with sensory disconnection from surgery) would signal to the anaesthetist to unnecessarily deepen the anaesthetic state increasing the risk of the side effects of anaesthesia. A marker of disconnection would have significant clinical utility to signify the lack of perception of surgery. Any insights afforded into the mechanisms of disconnection could lead to developments of improved anaesthesia or sedation in the critical care unit as well as development of further sleep aids for use in the community.

Patterns of brain activity during anaesthesia can be recorded using electroencephalography (EEG). EEG reflects the electrical activity of the brain, and is characterized by fundamental oscillatory patterns, including slow waves and spindles. A more complete understanding of these patterns will elucidate both normal brain function, and alterations in these patterns that may be sensitive indicators of disrupted brain function. This aim will be supported by studying a safe clinical sedative, dexmedetomidine (an alpha2 adrenergic agonist), that allows for rousable sedation to allow us to probe conscious state through questioning the participant. We will study the EEG correlates of conscious experience and lack of conscious experience under sedation. In order to obtain report of consciousness, we will wake patients from the sedated state and ask them questions to gain insights into the experience – if any – they were having at the time.

We have recently shown that arousals with unconsciousness were associated with loss of high frequency (beta power), and increased low frequency (delta power) in anterior and posterior cingulate cortex, key nodes of the default mode network.[4] At the scalp level, delta power at electrode Oz and Cz discriminated between conscious states (see Figure 1).[4]

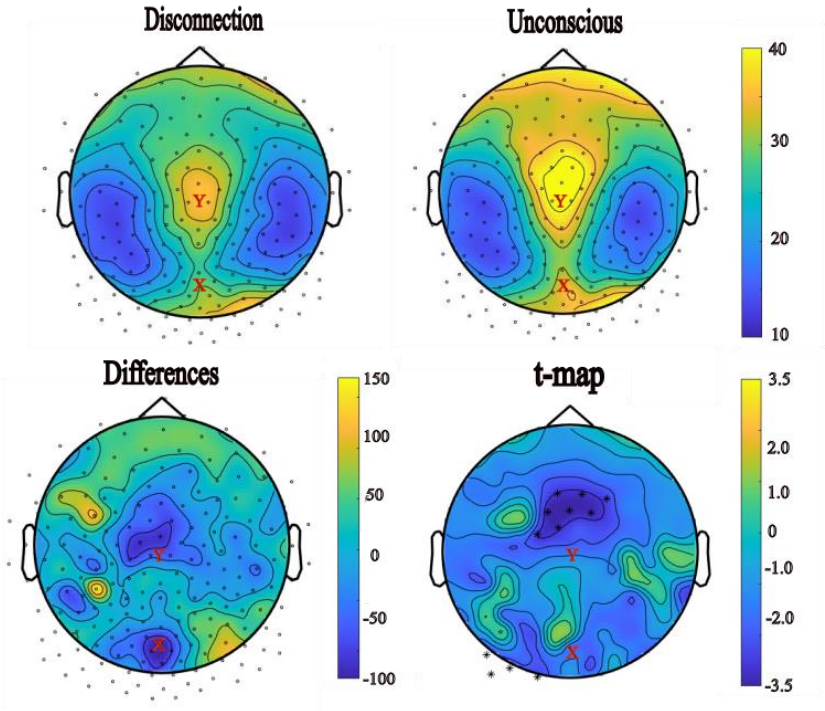


Figure 1: Comparison of delta power in Sensory Disconnection (Top Left) and Unconsciousness (Top Right) across the scalp. Bottom Left: Difference map between Disconnection and Unconscious. Bottom Right: t-map of differences between conditions. Asterixis represent significantly different clusters between conditions ($p < .05$).

Electrodes Oz and Cz are marked with an X and Y, respectively. Top Left and Right, and Bottom Left were thresholded for visualisation purposes only.

Anaesthetists use medicines to modulate brain activity patterns and hence modulate consciousness. However, if the critical feature underlying the loss of consciousness is disturbance of these oscillatory patterns, then a simple way to augment anaesthesia without inducing further drug-based side effects, could be to exogenously manipulate brain activity with low currents targeted to specific brain regions at low frequencies.[5,6] Meta-analysis shows that transcranial alternating current stimulation (TACS) is a safe way of modulating cognitive processing through modulating oscillatory activity in the brain.[7] We propose to combine low current low frequency TACS with a safe rousable sedative in a sham controlled randomized controlled trial to provide proof of concept that TACS may be used as an adjunct in the operating theatre in the future. If this is shown to work, then this could have two key benefits:

1. Reduce the risk from anaesthetic drugs, by supplementing them and reducing the total amount of drug required (with associated side effects)
2. Reduce the risk of anaesthetic awareness by manipulating brain activity in the key regions associated with consciousness.

Notably TACS is safe and uses currents that are between 10 and 100 times lower than clinically approved brain monitoring techniques used in theatre including somatosensory and motor evoked potentials. TACS technology is designated as non-significant risk.

Rationale for study of human volunteers

The rationale to study human volunteers has been carefully considered. To address the aims of this study there are no viable alternatives to studying healthy human volunteers:

Studying patients with pathologies necessitating surgery is imprudent as: (i) the worse health of surgical patients means that risks may be higher in patients with surgical pathology, (ii) anxiety associated with surgery may make participants less able to interact with study questions, and (iii) extending operating theatre times for

participants by 4 hours per participant will make running a "list" impractical, delaying care of other patients. Four hours of data collection ensures that we can slowly titrate sedatives, enhancing safety and maximizing the scientific gains of the study. It is also important to note that consciousness cannot be studied in animals as they cannot report their experience. The assumption that it can has severely hampered consciousness and cognitive research to date. Nonetheless, once we establish key neurophysiological correlates of human consciousness and cognition, we can then reverse translate that information and study the mechanisms of the neurophysiology in animals. The critical first step is to carefully define the relevant neurophysiology.

Safety

We also stress that the risk for human volunteers is extremely low. Using human volunteer sedation protocols, Dr. Sanders has conducted 41 sedations in human volunteers at the University of Wisconsin, with the only side effect being vomiting after ketamine (a known side effect for ketamine) – note ketamine will not be used in this trial.

Furthermore, as patient interaction is key for most of the study, over sedation leading to patient complications has never occurred in the 41 sedations conducted by Dr. Sanders. All sedations will also occur in equipped clinical environments, suitable for the care of patients with the supervision of an anaesthetist, ensuring the care of participants. We surveyed anaesthetists who have conducted similar sedation studies in volunteers and received descriptions regarding 404 volunteers. Note that some of these studies titrated the depth of anaesthesia to deeper levels than we wish to achieve (for example one targeted burst suppression in the EEG and another mandated placing a laryngeal mask airway device). Overall, these studies included 404 participants at Royal Victoria Hospital (Montreal), University Hospital (Liege), University of Pennsylvania, (Philadelphia), University of Washington (St Louis), University of Michigan (Ann Arbor), Oxford University Hospital (Oxford), University Medical Center Groningen (Groningen) and University of Wisconsin (Madison). In total, 404 participants were included in these studies and there were no reports of cardiac arrest, major medical complications, unanticipated respiratory arrest ("unanticipated" is included as one study specifically induced anaesthesia to a sufficient depth to place a laryngeal mask which typically induces respiratory arrest however this was part of the protocol) or death.

In order to advance the scientific understanding of consciousness under anaesthesia and the mechanisms of sensory perception, we need to undertake a series of human volunteer studies to define the neural correlates of these phenomena. Thereafter it will be possible to study the mechanisms, of these neural correlates, in animals. Studying the lighter doses of anaesthetics in this volunteer study may allow us to shift the emphasis to lighter doses of anaesthesia in the operating room, with anticipated small benefits to patients.

Overall, the study is safe, with nausea, vomiting and headache the only likely adverse events of the study. Nonetheless we will conduct these studies in an operating theatre with full access to resuscitation and clinical equipment as would be available for any other case undergoing anaesthesia.

1.2. RATIONALE FOR PERFORMING THE STUDY

This study will be a single-site, randomized, controlled, blinded study at the Royal Prince Alfred Hospital to examine changes in HD-EEG correlates of cognition and consciousness during waking and sedation. Our study will address the neural correlates of consciousness and modulate these neural correlates through low frequency TACS.

2. HYPOTHESIS

We hypothesize that delta band stimulation with TACS will reduce the incidence of dreaming under dexmedetomidine sedation, providing direct evidence of modulation of consciousness.

3. STUDY OBJECTIVES / AIMS

3.1. PRIMARY OBJECTIVES

The incidence of dreaming between TACS and sham stimulation under steady state dexmedetomidine.

3.2. SECONDARY OBJECTIVES

1. The incidence of disconnected conscious experience (dreaming) versus connected conscious experience (awareness of the external world) between TACS and sham stimulation and no stimulation.
2. The neural correlates of consciousness in anterior and posterior cingulate as identified by source reconstruction of beta/delta EEG power and other EEG analyses.
3. Differences in resting state power, evoked and induced responses between wakefulness, connected consciousness, disconnected consciousness and unconsciousness.
4. EEG responses and time to emergence following 15 minutes of TACS or sham.

4. STUDY DESIGN

4.1. DESIGN / STUDY TYPE

This study will be a single-site, randomized, double blind cross-over trial at the Royal Prince Alfred Hospital to examine changes in cognitive and EEG responses in different states of consciousness induced by the sedative drugs dexmedetomidine.

Participants will be screened for eligibility and then invited to attend a Sedation Visit at Royal Prince Alfred Hospital which will take approximately 8-9 hours.

The study activities are broken up into 4 stages which are described in Section 6.1.2 and the visual flow diagram.

4.2. EXPECTED PARTICIPANT NUMBERS

20 Participants

4.3. TIME PERIOD OF THE STUDY

Expected study duration: 1/9/2023 through 1/9/2024.

Below is an example of a basic Study Time Period:

Task	**Start Date**	**End Date**
Ethics Submission	May 2023	August 2023
Ethics Review and Approval	August 2023	October 2023
Advertising	October 2023	Ongoing

Recruitment	October 2023	October 2024
Conduction of consumer engagement activities	June 2023	Ongoing
Collection of data	October 2023	October 2024
Analysis of Data	October 2024	November 2024
Preparations of Reports	November 2024	December 2024
Publication Draft	January 2025	March 2025
Submission of Publications* and Final Reports	March 2025	May 2025

*We anticipate multiple EEG analytic publications will follow the results of the trial as we continue to optimise TACS stimulations and identify novel markers of the anaesthetic state.

4.5. CENTRES

Site Name/s	Royal Prince Alfred Hospital
Site Contact/Investigator	Professor Robert Sanders
Study Procedures	Screening, Recruitment, Pre-Study Assessment, Sedation Visit, Data Collection, Data Analysis, Adverse Event Reporting, Publication

5. STUDY PARTICIPANTS

5.1. INCLUSION CRITERIA

- Adults, ages ≥ 18 and ≤ 40 years old
- In good health, determined by the PI on the basis of medical history and a standard assessment for anaesthesia to be documented as part of the study record
- English Language Proficiency (suitable to provide informed consent and participate in research activities).

5.2. EXCLUSION CRITERIA

- Adults <18 years old or >40 years old
- Pregnancy confirmed on pregnancy test on day of sedation
- Use of recreational drugs
- Use of sedatives/sleeping medication within 24 hours prior to sedation visit
- Prescription for opioids (chronic or PRN) or other medications that cause sedation
- Contraindication to anaesthesia or allergy to study drug
- Difficult anaesthesia: American Society of Anesthesiologists Physical Status greater than 1, per the discretion of the PI. Examples of ASA>1 status includes, but are not limited to:
    - Any systemic disease present, such as diabetes, cardiac, pulmonary, or other acute or chronic disorder, or history of smoking
    - Narrow angle glaucoma
    - Abnormal airway examination
    - Any abnormality on physical examination that could increase anaesthetic risk
    - Snoring or sleep disorders including apnea
    - Antecedent pulmonary aspiration risk (e.g., history GI reflux, heartburn, hiatal hernia)
    - Adverse reaction or allergy with anaesthesia or other sedatives
    - Chronic medication use
    - History of difficult anaesthesia, laryngoscopy or intubation
    - Family history of difficulty with anaesthesia or sedation
    - History of vertigo, nausea or vomiting after anaesthesia
- BMI > 35
- Contraindication to HD-EEG for relative parts of the procedures.
- Exclusion from Dexmedetomidine:
    - Resting heart Rate<50 bpm
    - Known dexmedetomidine allergy
- People working in anaesthesia (such as anaesthetic registrars)
- People who are occupationally exposed to the study drugs.

Additional exclusion criteria on the day of sedation:

- Anything to eat or drink for the preceding 6 hours (excluding clear fluids)
- Anything to drink for the preceding 2 hours
- Any use of over-the-counter or recreational drugs (including alcohol or tobacco) within the preceding 24 hours
- Any use of opioid, sedative or sleep agents within the preceding 24 hours
- Recent change in health, including cough, cold, or fever
- Exposure to anaesthesia or sedation in the last 6 days

5.3 KEY ELEMENTS OF RECRUITMENT

1. Advertisements will be placed throughout the University of Sydney (pending approval) and placed in a limited number of community spaces (if approved by owner/management). Current plans are to limit advertising to the University and athletic facilities. This will be a one-page poster/flyer (see appendix).

2. Potential participants will contact a member of the research team by a designated telephone number and/or email address (provided on the advertising material). The participant will be contacted and provided verbal and written information, if happy to proceed with study and can meet the requirements, they will be contacted over the telephone for a screening. This will include eligibility, basic medical history and medication/drug list. All participants will be discussed with the principal investigator prior to arranging a study visit.
3. Participants with an ongoing professional relationship with the research team will not be eligible for the study. Participants with a previous medical relationship with any member of the research team will be reviewed for eligibility to ensure no ethical implications of the patient/doctor relationship.
4. Verbal consent will be obtained to perform eligibility screening questionnaire and written consent will be obtained prior to study activities. Participants will receive an electronic copy of the information sheet and eConsent will be used when feasible.
5. Participants who contact the research team will be provided with a patient information sheet as well as have ample time to discuss the trial prior to and during the sedation visit. Participants will have up to a month to consider their participation, discuss with family and discuss concerns/ask questions with research team.
6. As we are planning to advertise in the community, there is a risk that persons who are not eligible or cannot provide informed consent to contact the research team. All participants will be screened over the telephone and in person by senior investigators prior to continuing with any study procedures.

5.4 CONFOUNDERS

Due to the randomisation, blinding with sham stimulation there are limited confounders.

6. STUDY PROCEDURES

6.1. INVESTIGATION PLAN

Schedule of activities

	TELEPHONE SCREENING	SEDATION VISIT
TIME FRAME	Up to 30 days prior	
ELIGIBILITY SCREENING	X	X
INFORMED CONSENT	X	X
OBTAIN HISTORY & DEMOGRAPHICS	X	X
REVIEW OF CONCOMITANT MEDICATIONS	X	X
VITAL SIGN MEASUREMENT/PREGNANCY TEST		X
ANAESTHETIST REVIEW[1]		X
HD-EEG		X
Predictive Coding and Cognitive tasks		X
Auditory Stimuli		X
TACS		X
SEDATION+		X

PARTICIPANT REIMBURSEMENT	none	$200 for sedation visit

[1] History and Physical, Neurologic and Anaesthetic examinations for candidacy will be performed.

+Sedation will be with dexmedetomidine.

*None of the procedures in this study are considered to be standard treatments.

6.1.1 STUDY EQUIPMENT

Study Drug:

Dexmedetomidine

Dexmedetomidine is an approved clinical anaesthetic with a proven track record of safety. They will be used in a manner analogous to clinical care with appropriate safety measures, dosing, and treatments for side effects available. Contraindications to these drugs are rare but are a critical exclusion criteria. Sedation will be administered in areas with oxygen supply and suction available. Drugs will be administered consistent with RPAH safety guidelines with full safety monitoring and access to clinical resuscitation equipment. Normal saline 0.9% or Hartmann's solution will be available for fluid administration at a rate of 125 ml/hr or as determined by the anaesthetic doctor. An anaesthetist will be present throughout the study procedures until the patient is awake. Recovery from anaesthesia will occur under the supervision of trained clinical staff. All the sedative agents may provoke cardiorespiratory disturbance hence the drugs will be administered slowly, by an anaesthetist, in clinically approved environments. Full resuscitation equipment will be available throughout. The anaesthetist in charge may stop the study for participant safety reasons at any time and this will be conveyed to the DSMB.

Dexmedetomidine is an ICU sedative noted for its safety and lack of idiosyncratic drug reactions. All adverse effects are dose-dependent and hence dexmedetomidine will be given through slow intravenous infusion[8]. Dexmedetomidine is known for lack of respiratory depression and preservation of airway responses but is known to induce bradycardia and hypotension. Close attention to these parameters will be maintained and treated as per standard anaesthetic measures as required. If interventions are required, dexmedetomidine infusion will be stopped. Severe allergic reactions to dexmedetomidine have not been reported however, as with all anaesthesia care, full resuscitation equipment will be available. Allergic skin reactions to dexmedetomidine have been reported twice both with minimal effects.

Dexmedetomidine will be administered by infusion. As per the Australian Injectable Drug Handbook, typical dexmedetomidine infusions include up to 1 mcg/kg bolus over 10 minutes followed by infusion between 0.2-1.4 mcg/kg/hr until the desired OAA/S level is achieved. We will initially bolus 0.5 mcg/kg over 5 minutes for this study followed by 0.5mcg/kg/h infusion with dose increments targeting an OAA/S of 2 with clear verbal reports on wake ups. Incremental changes in infusion rate will be allowed to titrate to the participant's cognitive and physiological state including, for example, slowing bolus administration or boluses in an incremental fashion. Clinical judgment will be always used to prioritise participant safety. The anaesthetist will monitor the participant throughout to ensure they are safe, and the drugs are being administered at an appropriate rate as per clinical practice.

In prior experiments with human volunteers with dexmedetomidine, using deeper sedative dosing than in this setting and conducted by Prof Sanders, no subject required rescue medications or oxygen.

Clinical anaesthesia equipment: All equipment used in this study will have clinical approvals and be standard of care for use in patients. Oxygen, suction, fluid administration and resuscitation equipment will be available. In 41 human volunteer experiments conducted by Sanders, use of resuscitation equipment has not been required. Nonetheless, we will ensure clinical protocols are followed to ensure safety of all participants. Tetanic nerve stimulation will be delivered by the EZstim III nerve stimulating devices currently in standard clinical use during general anaesthesia at RPAH.

High-density (HD) EEG equipment: Identical in concept to previously existing EEG equipment. However, HD-EEG has many more individual electrodes placed across the scalp to allow superior spatial and temporal resolution in the analysis of the EEG signals. These systems are designed and marketed exclusively for research applications, and as such do not require clinical approval. However, these devices are not substantially different than the other EEG systems and are still considered non-significant risk devices.

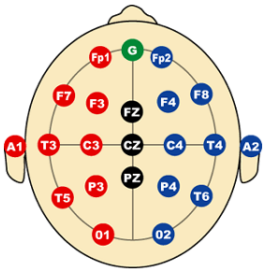
Auditory Stimulation: Devices that produce evoked auditory stimuli (an EEG response from an auditory stimulus) are described in 21CFR882.1900 as a Class II device (exempt from pre-market notification [510(k) clearance). Auditory stimuli will be produced on a standard personal computer through commonly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

Oddball Paradigm: We will use the "roving" oddball paradigm that has been used previously to study brain-damaged patients with altered levels of consciousness[9]. This paradigm has been well studied in other volunteer settings but not specifically in anaesthesia. The stimuli comprise a structured sequence of pure sinusoidal tones. The loudness of the tones will be set in each participant to a comfortable level, which will be maintained throughout the experiment. Within each stimulus train, all tones are of one frequency and are followed by a train of a different frequency. The first tone of a train is a deviant, which becomes a standard after several repetitions. This paradigm ensures that deviant tones and standard tones have exactly the same physical properties, differing only in the number of times they have been presented, which is varied pseudo-randomly between one and eleven. The frequency of the tones will vary from 500 to 800 Hz, in randomly selected integer multiples of 50 Hz. Stimuli will be presented binaurally via headphones for 20 minutes during wakefulness. The duration of each tone will be 70 ms and the inter-stimulus interval is 500 ms. 200 "standard sets" ranging from 1-11 tones/set (also resulting in 300 deviant tones) will be presented to each participant in each condition. We will compare the evoked response potential, time frequency responses and connectivity changes between the oddballs and standards, and their differences, across drugs and conscious states.

Additionally, we will use the oddball-by-omission paradigm[10]. The tones used are as specified above, however the pattern of the tones differs. In the omission paradigm, an expectation is established, for example by playing Tone A and Tone B continuously one after the other. Occasionally, one of these tones is skipped. When this omission occurs, a similar evoked response potential as from the deviant stimulus in the paradigm above can be recorded. In this case, the task will be presented similarly to above, with an omission occurring between 1 and 11 tones, resulting in 300 omissions in total. Presentation and analysis will be as above in other respects.

Transcranial Alternating Current Stimulation: TACS will occur using an Soterix MxN 33 channel transcranial alternating current stimulation device that is designed for non-invasive stimulation of brain function which has a long history of safety. Notably the stimulation currents are significantly lower than current stimulation techniques used in the operating theatre (e.g. 50mA is typically used for somatosensory evoked potentials and tetanic stimulation with up to 200mA used to drive motor evoked potentials).

TACS (or sham dependent on randomisation) will be performed 5 minutes prior to the wakeup protocol stage 3 (see section 6.1.3) being performed. The total amperes will not exceed 2 mA, and each stimulation period will be brief across different periods throughout the experience. Stimulation will be performed using five electrodes, four centred around Cz/Fz and one placed around Pz/Oz. These are located at the back and central area of the head. The placement of the electrodes makes a focal 'closed' circuit for stimulation around the given area.. Stimulation will be targeted to electrodes Oz/Cz/Fz using a five electrode montage



that has previously been shown to modulate default mode network activity and connectivity[11] and we have shown these regions are important for consciousness under dexmedetomidine sedation (Figure 1).[4] Stimulation will occur for 5 minutes at a time with a total of 1:15 stimulation time).

A Clinical Trial Notification will be submitted in conjunction with the Site-Specific Application for the use of this device.

The Biomedical Engineering Department will test and certify the TACS machine as safe for use in the hospital. Proof of this will be provided to the Ethics Committee on completion.

See Appendix 1 for TACS safety discussion.

Tetanic Stimulus: This will only be used in deeply sedated individuals (OAA/S 1), we will apply a tetanic stimulus for 10s at 50Hz 50mAmp to the forearm using a clinical nerve stimulator (EZstim III, model es400, Life Tech). Peripheral nerve stimulation is routinely used on emergence from anaesthesia to monitor return of muscle function. It is a moderately painful stimulus and hence we will not conduct it in wakeful individuals. However it has been used to assess rousability during anaesthesia previously[12].

Video Recording: Participants will be video recorded during the duration of each sedation appointment to ensure quality of research and to aid in analysis of the timing events following sedation. A written copy of the recordings will be made for use in the research and uploaded to the individual patient REDCap database. Video recordings will be stored in a secure departmental drive only accessible to the principal investigator and approved members of the research team for 15 years, and the video will be deleted from the video camera immediately following sedation.

6.1.2 STUDY ACTIVITIES

Initial Phone Screening/Assessment: Participants who contact the study team via number/email/enquiry form provided on advertising material will be contacted by a member of the research team over the telephone. A preliminary assessment will be done to determine eligibility and ensure the participant has adequate written and verbal information regarding the study. Eligible participants will be given time to consider their participation and provided contact information in order to ask questions and discuss the study. All potential participants will be discussed with the Principal Investigator to confirm eligibility/appropriate for study. If deemed eligible during this initial assessment, and confirming the participant has no medical issues or uses medications that would deem them ineligible, they will be asked to provide verbal consent or eConsent. Following this, they will be invited to attend Royal Prince Alfred Hospital on a specific date and time for the sedation visit. Information on fasting protocol, discharge information/instructions will be done prior to the visit over the telephone, they will also receive a reminder call on the day prior with instructions. If written instructions are required, an email will be sent at request of the participant.

Pharmacy:

The Investigational Drugs Unit at Royal Prince Alfred Hospital will provide dexmedetomidine for this study. A cost agreement and pharmacy SOP will be completed and uploaded as part of the site-specific application. Pharmacy staff will be alerted prior to the day of sedation visit, on the day the anaesthetic doctor will fill out a legal script for the participant for dexmedetomidine. This will be taken to pharmacy and the drug dispensed. The study drug will be labelled as per policy of the Investigational Drugs Unit and details will be recorded by both research staff and pharmacy staff to be kept with participant study documents. The Department of Pharmacy will be contacted prior to ethics and governance submissions and provide support during the application process.

6.1.3 SEDATION VISIT

The participant will be asked to attend RPAH at a pre-determined date and time for their sedation visit. On the day prior to their visit, they will be called by a member of the research team who will instruct them of the pre-study requirements and fasting instructions, ensure they have not met any ineligibility criteria, confirm they have arranged an escort and remind them to bring minimal personal belongings and leave valuables at home. They will be met at the hospital by members of the research team.

Assessment:

At the beginning of the visit, an anaesthetic doctor and the participant will have an informed consent discussion and participants will be given time to ask questions and discuss the study further if required. If all criteria for inclusion are met, and the participant still agrees to consent, an anaesthetic doctor will collect information on their medical history, do a brief physical assessment and standard anaesthetic examinations (airway assessment/adequate venous access). Vital signs will be measured, and a review of concomitant medications and drug screen will be performed. This information will be documented on a standard Anaesthetic Chart used for procedural sedation/anaesthetics (see appendix). If during any of these assessments, the anaesthetic doctor feels the participant is not appropriate for the study, they will not progress to the sedation activities. Female patients will have a pregnancy test on the day of the sedation to confirm they are not pregnant.

Sedation Set-up:

All sedations will occur in an operating theatre with full access to standard clinical monitoring, equipment, and rescue medications as well access to resuscitation teams.

Once in the procedural area, participants will have the EEG cap placed and a 20-gauge intravenous catheter will be placed for drug delivery. Supplemental oxygen will be provided at 0-10 L/minute via nasal cannula as required. The participant will receive normal saline 0.9% or Hartmann's solution at 125 ml/hr during the sedation visit. The participant's ECG, non-invasive blood pressure, SaO2, and exhaled CO2 will be monitored continuously. Before each experiment, the anaesthesia machine and drugs will undergo routine pre-anaesthetic scrutiny, and the availability of resuscitation drugs and equipment will be confirmed and documented.An anaesthetist whose sole responsibility is participant welfare will administer the sedative (dexmedetomidine) and will be responsible for monitoring physiological status and documenting vital signs on the standard anaesthesia record. Level of consciousness will be assessed using the 5-point OAA/S scale. The level of 1 corresponds to "no eye opening or coherent response to verbal command or mild physical stimulus (mild shaking or prodding)". At this level of responsiveness, airway patency and ventilation are maintained in most, if not all, patients. Patients will not be routinely intubated for this study.

Study activities will be divided into four stages – see below for details on each stage and estimated time requirements.

Stage 1: Resting/Wakeful State (1 hour)

Prior to administering sedation resting/wakeful state data will be collected. During this stage EEG data will be recorded on the attached computer and time stamps/notes will be documented by the anaesthetic doctor and research team (see appendices for CRF outlining data recorded).

Initially baseline EEG data will be recorded with eyes open and closed. Following this the participant will be randomised live to either resting (no stimulation) or oddball (auditory stimulation). Over the course of this hour, they will have 7 x 5-minute blocks of resting state and 3 x 7-minute blocks of auditory stimulation. Randomisation will occur at the beginning of each block so that there is no specific pattern or opportunity for bias. A total of 10 wake-up cycles will take place and following each wake up the participant will be asked some basic questions about their experience.

The following questions will be asked following each wake up in all stages of the study:

1. What was going through your mind before awakening?

2. Do you think you were awake/dreaming/unconscious?
3. Were you asleep or awake? (Visual Analogue Score 0-6)
4. Did you hear the tones/words? Were you aware of your environment?
5. Anything else to report? Did you see faces? Or movement?
6. Did time pass? Can you estimate how long passed since we last spoke to you?

Stage 2: Sedation Start

During this stage, dexmedetomidine will be titrated initially to an OAA/S Score of 2 (see table below), as per the dosing regimen outlined in section 6.1.1.Baseline data will be recorded on the EEG machine with eyes open for a period of time, followed by eyes closed. Following this the participant will go through a series of 10 wake ups as described in stage 1. They will be randomised to resting or oddball at the beginning of each wake up. Following each wake up they will be asked basic questions about their experience. At these doses we hypothesize that altered predictive coding will be associated with changes in alpha power and connectivity in the EEG.

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

Image of Soterix electrode Cap

The questions outlined in stage 1 will be asked following each wake-up in stage 2.This will provide information about whether they were conscious and what they were conscious of. Specifically, we will record whether they were conscious of their environment (connected consciousness), dreaming (disconnected consciousness, DCON), conscious but cannot remember details (DCON without recall), unconscious (no report). We will also ask whether they noticed time passing. We anticipate the questions will take approximately 30 seconds. The series of 10 wake ups with questioning will take approximately 1 hour, however this could be slightly longer, taking into account time to fall asleep and wake up.

Stage 3: TACS/Sham (3 hours)

During this stage we will then randomise the individual to 5-minute blocks of sham (no stimulation) or TACS stimulation targeting the occipital electrode Oz, and Cz at 2Hz.[11] Stimulation at electrode Oz has been shown to modulate the midline default mode network, that are the key nodes of the brain that we have identified as associated with consciousness.[11] As our prior experimental data suggest that unconsciousness is associated with increases in delta power around Pz/Oz, Cz/Fz electrode locations, we will stimulate at each of these locations. After the 5-minute period, the participant will then be woken and asked to report their conscious experience. The process will then be repeated for 3 hours (aiming for 15 wake ups following sham and 15 wake ups following TACS).

TACS Stimulation: Participants will be randomised live to TACS or sham at the beginning of each wake up (15 of each), stimulation will occur for 5-minute periods.

The same questions outlined in stage 1 will be asked following each wake- up in stage 3.

Stage 4: End Sedation (1 hour)

At the beginning of this stage participants will be randomized to 15 minutes of TACS or sham. Three minutes following the stimulation block, dexmedetomidine will be terminated and time to spontaneous wake up will be measured, followed by verbal interview.

TACS Stimulation: Participants will receive 15 minutes of TACS or sham stimulation.

Recovery:
Participants will be observed in the clinical environment by trained anaesthetists or registered nurses until they are judged by the anaesthetist to be ready to move to be discharged home. We anticipate the full recovery from the sedatives to take at least two hours. Orders for post-sedation care will follow standard post-sedation hospital guidelines for patients after surgery.

Participants will be discharged by the clinical anaesthetist directly or by delegation to a trained nurse according to safe discharge criteria. Participants will be given a copy of instructions (RPA "Perioperative Unit General Discharge Instructions" Appendix X) and contact information in the event that complications arise after leaving the hospital. Additionally, a letter detailing their sedation visit procedures will be provided in the instance it is required by their GP or healthcare worker in the days following their sedation visit. As per hospital policy, they will be required to have an escort collect them and accompany them home. Patients will be instructed not to drive or operate machinery, and not to consume other drugs with central nervous system activity within 24 hours of drug-induced sedation as per standard instructions after procedural sedation.

Sedation Visit Follow-Up
Participants will be called by study staff the day following their sedation appointment. During this phone call, the participants will be asked about their recovery from sedation and any adverse events (headache, nausea, dizziness etc.) will be recorded. This information will be documented on a post-procedure adverse event form in the REDCap database.

A week following their sedation visit participants will be emailed or text an optional Research Participant Feedback Survey via a REDCap link.

6.2. STUDY PROCEDURE RISKS

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

None of the procedures included in this study are considered to be therapeutic or diagnostic, as detailed for each specific procedure. Each of the procedures has minimal risks associated with them, and have been employed successfully, without incident, by our research group.

6.3. POTENTIAL RISKS

DRUGS

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

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

Intravenous Catheters:
The attending anaesthetist will place intravenous catheters on the day of visitation (local anaesthetic may be used). Nevertheless, there may be some discomfort to participants upon insertion, and there may be a slight risk

of bruising, and inflammation after removal. In normal participants subcutaneous infiltration and induration are very rare complications and are easily managed with warm packs and oral analgesics.

Respiratory depression / loss of patent airway:
Because the anaesthetics will be administered incrementally under close observation, we expect that if respiratory depression occurs it will be limited in depth and duration. In 41 prior sedations by Dr. Sanders no patient experienced respiratory obstruction requiring airway support. However, if required, supplemental oxygen will be provided, a reposition of the airway (i.e., jaw thrust) and assisted ventilation with bag and mask will be sufficient to return the participant to full ventilatory control within several minutes. If greater assistance is required, a standard oral, nasal and laryngeal mask airways will be immediately available. Caregivers are skilled in their use. Full resuscitation equipment will also be available if required. Instances of oxygen desaturation that require airway support or assisted ventilation will be reported as an adverse event and included in safety monitoring reports.

Pulmonary aspiration of gastric contents:
Participants who have fasted for 6-hours are at very low risk for pulmonary aspiration. Participants will be closely monitored for signs of nausea and vomiting, and the experiment will be terminated in the event of emesis. Participants will be sedated in the operating room on tables allowing positions that reduce the risk of aspiration even further, and a fully operational suction apparatus will be available at all times. During recovery, participants will be closely observed for signs of aspiration, and instructed in the steps to take if signs of aspiration appear after discharge.

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

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

*The PI and colleagues have established an approach to sedation using dexmedetomidine that allows for frequent wake ups and low risk of prolonged sedation as outlined in Casey et al, 2022. These are described in the drug dosing schedule above.

Drug allergy and other side effects:
Allergies to dexmedetomidine administration are extremely rare. Patients with known allergy will be excluded. While a number of side effects have been reported with acute and chronic use, they are very uncommon. Common or serious risks include a residual 'hangover', such as sleepiness, and impaired psychomotor and cognitive functions that may persist into the next day and may impair the ability of users to drive safely.

Participants with known allergies to any medications used to counteract common side effects will be noted and given alternative options or interventions at the discretion of the anaesthetic doctor, in line with standard care.

Transcranial Alternating Current Stimulation:
TACS is used in in research as its stimulation techniques can non-invasively modulate neural activity without causing pain. All known side effects are transient. The currents used are weak but there is a small risk of minor discomfort, itching, tingling, redness at the site of the electrodes. Additional side effects associated with current stimulation techniques include headaches, perception of phosphenes and dizziness.[13]

Risks associated with loss of confidentiality

There is a risk that information recorded about participants will be shared with people who would not normally have access to this information.

Unknown risks

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

6.3.1 MITIGATION OF RISKS

An anaesthetist will ensure that participants fit the inclusion criteria for the study and patients deemed unsafe to participate will not be enrolled. The anaesthetist will be present throughout the study to ensure safety at clinical standards. If it is judged the patient is at risk from continuation of the study, the study will be stopped. Patients who are at risk from seizure induction will be excluded.

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

Mitigation of risks associated with Sedation: The anaesthetist will be present throughout the study to ensure safety at clinical standards including administration of required medications and fluids to maintain participant safety. Common side effects such as hypotension and bradycardia may occur with the use the use of dexmedetomidine. Any side effects will be treated following standard care practices at the discretion of the anaesthetist. Clinical grade anaesthesia and resuscitation equipment - including airway support equipment and ventilator- will be available throughout the study. Participants will be instructed not to drive or operate heavy machinery and must be accompanied by someone who can take them home or schedule a ride from another service. The study coordinator will verbally confirm this with the participant. The study coordinator will also instruct the participant to be accompanied overnight and verbally confirm this will happen with the participant.

Mitigation of risks associated with insertion of cannula: An experienced anaesthetist will insert the cannula. The site will be checked and dressed prior to discharge.

6.4. POTENTIAL BENEFITS AND RISK-TO-BENEFIT RATIO

No direct benefit will accrue to participants. We anticipate that society may benefit from the possible development of a new diagnostic technique (i.e., a monitor of sedation and anaesthesia), and because information may be obtained regarding the mechanisms of conscious experience that has important ramifications for detecting/preventing consciousness during anaesthesia and sedation and in neurological conditions such as unresponsive wakefulness syndrome and coma. Furthermore, in our recent ConsCIOUS study, 11% of young adult patients during routine general anaesthesia were aware of intubation (connected consciousness). Half of these people reported pain. This is a good example of why it is important for us to understand the mechanisms and markers for conscious experience that is related to the external world (connected consciousness). To identify the markers and mechanisms of connected consciousness, it is important to perform "within state" experiments such as probing conscious experience during sedation, as we plan here. If we can modulate conscious state with TACS, then we will have potentially identified a non-invasive method to reduce the risk of awareness under anaesthesia. These potential benefits go beyond anaesthetics and may inform diverse fields from the disorders of consciousness (such as coma or delirium) to sleep.

6.5. PARTICIPANT RECRUITMENT AND SCREENING

Who will make initial contact with participants?	Participants will contact research team via contact details included in advertising material.
What will happen with the information collected during the screening process?	If a potential participant agrees to the screening phone call and is deemed ineligible to participate or refuses to consent this will be documented in a screening log. Any personal information and contact details will not be saved in the screening log- only anonymous data on reason for ineligibility or declined.
Who will perform the consent process? How will this be carried out?	Informed consent will be completed by a trained member of the research team. Participants will be provided with verbal and written information and given time to review/discuss/question prior to signing consent. The research team member obtaining consent will be responsible for confirming the participant is able to give informed consent.
Will participants be consented verbally/explicitly/using eConsent?	Electronic consent will be obtained when feasible, otherwise verbal consent will be obtained over the phone and written consent will be obtained during the sedation visit.
Will participants be given a specific time period to consider participating?	YES, Participants will have up to 30 days to consider participation and multiple opportunities to discuss with a member of the research team.
Review of existing databases or databanks (please identify the database/databank and the custodian)	N/A

Review of clinic files (please include who will be reviewing these files, for example a research coordinator).	Data forms collected during the visits will only be viewed by members of the research team included on the ethics application.
Advertisements (please include where the advertisement will be placed for example, in a newspaper, poster in a clinic or hospital foyer, radio announcements, website etc.)	Advertisements will be placed in common areas of The University of Sydney (pending approval) and other community areas. All advertisements will be approved by ethics prior to use.
Information Letter to Medical practitioners	YES, a letter of participation including details of the sedation visit will be provided to the participant at the end of the sedation visit. This can be given to their GP or used to inform subsequent healthcare workers of their participation.

6.6. PARTICIPANT ENROLMENT

Potential participants will be enrolled into the study after the informed consent process has been completed and the participant has been assessed to meet all the inclusion criteria and none of the exclusion criteria.

Participants will be allocated a study ID which will be used on data collection forms.

6.7. INFORMATION AND CONSENT

Participants will provide informed consent and sign the consent form.

Research staff obtaining informed consent will ensure participants are able to provide informed consent. Participants will provide written consent via an electronic form or in person.

6.8. RANDOMISATION PROCEDURE

The participant will be randomised live on the day of their sedation and will occur prior to the activity. Randomisation will occur in all four stages of the study and be done by the randomisation module in REDCap (SLHD iteration).:

1. In Stage 1 and 2 – Participants will be randomized prior to each wake up cycle (5-7 minutes) to either resting state (no stimulation) or oddball (auditory stimulation). They will have a total of seven 5-minute resting state cycles and three 7-minute oddball cycles. Randomisation will be done live to ensure no pattern or pre-determined bias of the patient to resting/auditory stimulation. The order of activity will be determined by randomisation.
2. In Stage 3, participants will be randomised to either TACS or sham stimulation for 5-minute periods. This stage is a cross-over design and randomisation will occur at the beginning of each cycle. There will be a total of 30 wake ups (15 TACS and 15 sham), the order of the activities will be determined by the randomisation.

3. In stage 4, they will be randomised to either 15 min TACS or sham stimulation (no cross over n=10 per group).

6.9 END OF STUDY TREATMENT/WITHDRAWAL PROCEDURE

Participation in the study will conclude after the sedation visit. A member of the research team will contact the participant via telephone on the following day to ask about their recovery and note any adverse events. One week following their sedation visit an optional REDCap survey will be emailed to the participants. This survey will address their experience as part of the study and provide feedback to the research team.

6.10. PATIENT WITHDRAWAL

Participants may withdraw at any time at their discretion. In the unlikely event that safety concern arises, the study subject will be withdrawn from the study and dexmedetomidine ceased. This participant's data will be reported to ensure explicit reporting of all safety data. Any withdrawals due to adverse events and protocol deviations or violations will be reported the local HREC and governance office.

Withdrawn participants will not count as part of the total recruitment numbers.

7. OUTCOMES

7.1. DEFINITION OF OUTCOMES

Connected consciousness – wake report describing conscious experience of the sensory environment while on dexmedetomidine

Disconnected consciousness – wake report describing conscious experience of a dream and the absence of sensory environment while on dexmedetomidine

Unconsciousness – wake report describing no conscious experience while on dexmedetomidine (e.g. mind was empty, blank, nothing there

Time to emergence – time from study drug cessation to spontaneous eye opening.

8. INVESTIGATIONAL MEDICINAL PRODUCT(S)

8.1. DESCRIPTION OF INVESTIGATONAL PRODUCT

a. Generic name – Dexmedetomidine

b. Brand - Precedex

c. Strength – 200mcg/2mL vial

d. Form (i.e. tablet, injection) - injection

e. Route (i.e. oral, IV) - IV

f. Supply – Investigational Drug Unit at RPAH will supply- a cost agreement and dispensing protocol will be provided to Ethics.

g. PBS and ARTG listing status (for Australian studies)- ARTG ID **379412**

8.2. PHARMACOKINETICS

Dexmedetomidine is an α_2-adrenoceptor agonist with sedative, anxiolytic, sympatholytic, and analgesic-sparing effects, and minimal depression of respiratory function.

8.3. ADMINISTRATION AND DOSE

Dexmedetomidine will be administered by infusion. Typical dexmedetomidine infusions include up to 1 mcg/kg bolus followed by infusion between 0.2-1.4 mcg/kg/hr until the desired OAA/S level is achieved. We will initially bolus 0.5 mcg/kg for this study followed by 0.5mcg/kg/h infusion with dose increments targeting an OAA/S of 2 with clear verbal reports on wake ups. Incremental changes in infusion rate will be allowed to titrate to the participant's cognitive and physiological state including, for example, slowing bolus administration or bolusing in an incremental fashion. Clinical judgment will be used at all times to prioritise participant safety. The anaesthetist will monitor the participant throughout to ensure they are safe, and the drugs are being administered at an appropriate rate as per clinical practice.

8.4 HANDLING AND STORAGE OF STUDY DRUGS

Study drugs will be stored in a temperature-controlled room/cupboard, monitored by pharmacy staff. The Investigational Drugs Unit of the RPAH pharmacy will be responsible for the supply and dispensing of the study drugs.

8.5 FUNDING

Costs of drugs and patient reimbursement will be covered by research funds raised in the Department of Anaesthetics.

8.6 CTN

A Clinical Trial Notification for both dexmedetomidine and the Soterix MxN 33 TACS device will be competed and submitted to the TGA during the Site-Specific Application process.

9. DATA COLLECTION

9.1. PARTICIPANT REGISTRATION

Participants will be enrolled in the trail and given a study ID. A Master Code Sheet will be kept separately in REDCap with demographic information (SLHD iteration).

9.2. FORMS AND PROCEDURE FOR COLLECTING DATA

REDCap (SLHD iteration) will be used for data collection. Any paper CRFs used by anaesthetics for documentation of data during the telephone screening and sedation visit will be transcribed to REDCap. Transcribed data will be double checked by 2 members of the research team prior to completion. No one except trained and approved members of the research team will have access to the REDCap database or paper forms.

Details on data management and storage can be found on the Research and Data Management Plan included in the Ethics submission.

9.3. CASE REPORT FORMS

See appendices for Case Report Forms

10. STATISTICAL METHODS

10.1. SAMPLE SIZE ESTIMATION

The sample size estimate assumes a beta of 0.8 and a two-side alpha of 0.05. Based on prior data, we expect that 60% of the wake ups from dexmedetomidine will be associated with disconnected consciousness during sham stimulation (assuming no difference from 'no sham') and we assume that TACS will reduce the incidence of disconnected consciousness by one third (absolute decrease of 20%, standard deviation of 20%). Based on these assumptions we require a total of 20 patients (assuming 15 wake-ups with TACS stimulation and 15 wake ups with sham stimulation) and assuming a 20% loss to follow up.

Missing data

We anticipate missing data from some participants not being able to complete the tasks under the sedation due to side effects.

10.2. STATISTICAL ANALYSIS PLAN

Stage 1 consists of collecting baseline EEG response to auditory evoked data and resting state data. This will be compared to the EEG responses collected from the same conditions in Stage 2 while participants are under light sedation. This comparison will consist of planned contrasts between the correlation of evoked responses to auditory stimuli under no- and light-sedation, analysed using standard EEG processing and cluster-based statistics as implemented in Fieldtrip. Resting state data will be compared between Stages 1 and 2 in terms of prediction of state, as performed using machine learning classification techniques.

The first primary comparison of interest is between the incidence of dreaming and unconsciousness during baseline (Stage 2) to either sham stimulation or TACS. To analyse this, we will use linear mixed effects models with random effects for participants, and fixed effects for Stage (2 or 3) and Stimulation (Real or Sham). We hypothesize an interaction effect, in which stimulation will increase the rate of disconnection in Stage 3 and have no effect in Stage 2 (in which no stimulation is given and thus which condition the subject is randomised should have no effect).

The second primary analysis is of Stage 4 data. Stage 4 will be analysed using a linear mixed effects model which attempts to predict the time-to-awakening, with subjects as random effects, and whether the participant had been given real or sham stimulation as a fixed effect. This model may also include baseline rate of disconnection per subject, and whether they were given real or sham stimulation in Stage 3.

We will include all available data in the models (e.g. linear mixed effects models with random effect for participant) and conduct sensitivity analyses with just paired data.

11. QUALITY CONTROL AND ASSURANCE

11.1. CONTROL OF DATA CONSISTENCY

All data transcribed to REDCap will be checked by the Anaesthetic Research CNC for completeness and consistency.

11.2. AUDITS

Internal audits of transcribed data will occur at interims during study activities. External audits may be conducted at the discretion of the DSMB or TGA.

11.3. PROTOCOL AMENDMENTS

All protocol amendments will be submitted to the local HREC for review and approval prior to implementation.

12. ETHICS

12.1. INVESTIGATOR AUTHORISATION PROCEDURE

The Ethics application will be submitted to the local HREC (RPAH Zone) for approval, following Ethics approval a Site-Specific Application will be submitted and Clinical Trial Notification to the TGA. Additionally a Cost Agreement will be finalised with the Investigational Drugs Unit and a Material Transfer Agreement uploaded with the SSA.

No study activities, advertising material or documents will be completed/used until full approval has been granted.

12.2. PATIENT PROTECTION

As per the National Statement on Ethical Conduct in Human Research (2007) (the National Statement) and the CPMP/ICH Note for Guidance on Good Clinical Practice, the research team will ensure all activities are conducted in a safe and ethical manner. If at any time a member of the research team, participant or colleague raises concern with the safety of the study, recruitment will be paused, and the issues will be addressed. Any safety concerns or amendments to the protocol to improve participant safety will be submitted the local HREC for approval.

All measures will be taken to ensure participants personal information remains protected, including the use of study IDs and use of separate Master Code Sheets.

12.3 PARTNERING WITH CONSUMERS

The National Health and Medical Research Council (NHMRC) have published a statement on Community Involvement in Health (September 2016) co-authored by the National Health and Medical Research Council (NHMRC) and the Consumers Health Forum of Australia (CHF). This statement aims to guide research institutions, researchers, consumers and community members in the active involvement of consumers and community members in all aspects of health and medical research. The research projects run through the department will benefit from consumer and community involvement by ensuring research is relevant to community needs, improve awareness of medical research and improve facilitation of research outcomes to daily practice. Additionally, researchers will have increased opportunities for recruitment and improve the quality research.

The Department of Anaesthetics and research staff understand the value that consumer involvement adds to medical research and is committed to facilitate consumer engagement in ongoing clinical research. The research team within the Department of Anaesthetics will actively engage and support community engagement through a variety of activities throughout the life of this trial and future clinical studies. In order to put this into practice the PI and research coordinator have identified opportunities for future consumer engagement:

- Anonymous poll of adults 18-40 within the community to gage interest, receive feedback on the study design, and address potential questions and concerns.
- Creation of a participant database (with consent) that can be used for clinical trial feedback and quality assurance following the completion of the study visits.
- Identification of a consumer representative to recruit for ongoing involvement during the lifespan of the project.
- Research Participant Feedback forms will be emailed to participants in the week following their sedation study. Survey will be administered via emailed REDCap link. This survey will provide information and feedback on participants experience in this trial, attitudes towards the study subject and guide design of future trials.

(Statement on Consumer and Community involvement in Health and Medical Research, National Health and Medical Research Council (2016), Consumers Health Forum of Australia)

13. SAFETY

13.1 ADVERSE EVENT REPORTING

Adverse event (AE)

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

Serious adverse event (SAE)

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

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

Unanticipated problem (UP)

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

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

a. Reporting period

Any adverse events or serious adverse events will be reported within the timeframes outlined by NHMRC Safety Reporting Guidelines.

b. Severity assessment

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

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

c. Causality assessment

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

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

d. Procedures for recording and reporting adverse events

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

e. Other reportable events

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

Event	Definition	Reporting
Breach of confidentiality	The exposure of any study information or communications directly related to a study participant to anyone not named as study staff or the release of a study participant's identifiable information to study staff who were not specified to receive such information in the protocol or HREC application.	Treat as major deviation
Protocol deviation	A deviation is an incident involving a departure from the HREC-approved protocol in the actual conduct of the study. Deviations may result from the action of the participant, investigator, or staff.	See below

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

Reporting by study site to local HREC
The study site will follow their local HRECs guidance for reporting all events to the local HREC.

13.2. SERIOUS ADVERSE EVENT REPORTING

All serious adverse events should be reported immediately to the HREC/sponsor. The reports should be followed by a detailed written report. Follow-up reports should identify the participant/s by unique code assigned to participants (rather than by name).

13.3. DATA SAFETY AND MONITORING BOARD (DSMB)

A Medical Monitor will be appointed who may perform oversight functions (e.g., observe recruitment, enrolment procedures, and the consent process for individuals, groups or units; oversee study interventions

and interactions; review monitoring plans and reports; and oversee data matching, data collection, and analysis) and report the observations to a DSMB.

The Medical Monitor/DSMB shall have authority to stop a research protocol in progress and take whatever steps are necessary to protect the safety and well-being of human participants.

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

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

We propose the Medical Monitor will Chair a Data Safety Monitoring Board of three anaesthetists who are external to the RPA Department of Anaesthetics. These individuals will be selected after the external peer review of the proposal. The proposed individuals for external peer review are:

Prof Paul Myles, Monash University, p.myles@alfred.org.au

Prof David Story, University of Melbourne, dastory@unimelb.edu.au

Dr. Peter Schuller, Cairns Base Hospital, peterjschuller@gmail.com

Dr. Stefan Dieleman, Westmead Hospital, stefan.dieleman@sydney.edu.au

Dr Matt Doane, Royal North Shore Hospital, Matthew.Doane@health.nsw.gov.au

The DSMB will meet after the first participant is recruited and sedation is complete. It will then meet every 5 participants recruited. Additional ad hoc meetings will be convened if safety issues arise.

Prof Sanders will attend an open meeting of the DSMB to provide information and will be excluded from the DSMB meeting for a confidential portion of the meeting while the study is discussed.

13.4. EARLY TERMINATION

If early termination of the trial is deemed necessary by the Principal Investigator, this will be discussed with the DSMB and HREC. A final study report and letter to participants will be completed as per local HREC guidance.

14. BLINDING AND UNBLINDING

The participant will be blinded to stimulation by either TACS or sham.

The adjudicator of conscious state and the person determining eyes open will also be blinded to TACS or sham.

15. CONFIDENTIALITY AND STORAGE AND ARCHIVING OF STUDY

Data collection is the responsibility of the research staff of Royal Prince Alfred Hospital Department of Anaesthetics under the supervision of the Principal Investigator. The Principal Investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

The SLHD software REDCap (Research Electronic Data Capture) will be used to capture and store research data. A separate Master Code Sheet will be used for identifiable patient data. The participant Study ID will be linked to a de-identified record within a separate research data project in the REDCap system using their unique participant Study ID. The Master Code Sheet will only include basic participant identifiers required for the study and no additional personal information will be collected.

Any paper documents will be stored in a locked cupboard in the locked Department of Anaesthetics RPAH and managed by the Research CNC. Any transcribed data from paper to REDCap will be checked by two members of the research team to ensure accuracy and completeness.

Only trained and approved members of the research team will have access to the Master Code Sheet and REDCap database.

Data analysis will occur using de-identified data only.

Data will be kept for 15 years.

16. TRIAL SPONSORSHIP AND FINANCING

This study is sponsored by the Sydney Local Health District and funded internally by the Department of Anaesthetics at Royal Prince Alfred Hospital.

16.1 COMPENSATION OF TIME

Participants will not be paid for this study but to compensate for their time they will receive a $200 voucher on completion of the study visit.

17. INDEMNITY

This is an investigator-initiated study and indemnity/insurance is covered by the public health sector.

18. COMPENSATION

If a participant suffers any injuries or complications as a result of the research project, they will be advised to contact the study team and will be assisted with arranging appropriate medical treatment. If participants are eligible for Medicare, they can receive any medical treatment required to treat the injury or complication, free of charge, as a public patient in any Australian public hospital. In addition, patients may have a right to take legal action to obtain compensation for any injuries or complications resulting from the study. Compensation may be available if their injury or complication is sufficiently serious and is caused by unsafe drugs or equipment, or by the negligence of one of the parties involved in the study (for example, the researcher, the hospital, or the treating doctor). Patients do not give up any legal rights to compensation by participating in this study."

19. CONFLICTS OF INTEREST

The Principal Investigator and Associate Investigators of this trial have no conflict of interest to declare.

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21. APPENDICES

1) TACS Safety

The majority of side effects are itching, tingling, rarely dizziness, and redness under the electrode cite (Matsumoto & Ugawa, 2017) with no persistent side effects have been reported following TACS according to this review article. Prior to study activities, an anaesthetic doctor and member of the research team will fully assess the participant for any risk factors including sensitive skin, allergies, pre-existing conditions that may cause dizziness etc.…This will further reduce the probability of side effects. This method of stimulation is considered so safe that it has been used in home-health settings without doctor supervision (Eapen et al., 2017). Further, a recent review of 1429 participants (De Koninck, Brazeau, Guay, Herrero Babiloni, & De Beaumont, 2023), did not report significant negative side effects in patients, including some with stimulation intensities up to 6 mA, 3-times higher than the max stimulation intensity we will use. In this regard, we believe it unlikely for our proposed method to cause any direct harm.

In terms of the frequency chosen, 2Hz has been shown to have a similar side effect profile to other frequencies used (Raco, Bauer, Olenik, Brkic, & Gharabaghi, 2014).

References:

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List all appendices and submit as stand alone documents i.e.:

2) Anaesthetic Record
3) Data collection sheet/Case Report Form
4) Master Code Sheet
5) Discharge Instructions and Letter to GP
6) Telephone Screening Script
7) Verbal Consent Form
8) Patient Information Sheet and Consent Form
9) Advertisement Flyer
10) Research Participant Survey