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Chief Investigator Signature:

Conflicts of interest

Dr Warnaby, Dr Richard Rogers, Dr Saad Jbabdi and Professor Tracey (and others) are inventors on a patent application filed by Oxford University Innovation Ltd. (formerly Isis innovation) on Perception Loss Detection (PCT/GB2013/051445, US61/746975) using slow wave activity saturation (SWAS) for depth of anaesthesia monitoring.

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor and host organisation (University of Oxford), the Investigator Team, HRA, the LUMINOUS consortium and associated Ethical Advisory Board (EAB), and members of the Research Ethics Committee, unless authorised to do so.

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1. SYNOPSIS

Study Title	Brain imaging of anaesthesia	
Internal ref. no. / short title	[PID13238] Brain imaging of anaesthesia	
Study Design	A patient study will be performed to optimise delivery of anaesthesia to the endpoint of slow wave activity saturation using real-time feedback of electrical brain activity. Brain imaging (and sleep recordings) in healthy volunteers at baseline, loss of behavioural responsiveness and slow wave activity saturation during sevoflurane anaesthesia.	
Study Participants	Patients and healthy volunteers aged 18-60 years with an American Society for Anesthesiology (ASA) score 1 or 2	
Planned Sample Size	Up to 30 patients and 30 healthy volunteers	
Planned Study Period	3 years	
	Objectives	**Outcome Measures**
Primary	1. Assess resting and functional thalamocortical connectivity at loss of behavioural responsiveness (LOBR) and slow wave activity saturation (SWAS)	1. Changes in connectivity of sub-regions of thalamus with the cortex at LOBR and SWAS compared with the baseline (no anaesthesia) condition during resting and task-based functional imaging scans.
Secondary	1. Demonstrate that it is possible to titrate anaesthesia to SWAS on individual basis using real-time EEG feedback	1. Achieve stable SWAS endpoint on an individual basis within 15 minutes
	2. Demonstrate that individuals held at SWAS are unresponsive to pain and have no conscious content as assessed by the isolated forearm test	2. Significant reduction of FMRI thalamocortical brain activity in response to pain at SWAS compared with loss of responsiveness No behavioural response (as assessed by hand open/close) to verbal request when held at SWAS
	3. Explore common mechanisms and influence of sleep on anaesthesia	3. Within-subject correlation of EEG power at SWAS under anaesthesia and maximum EEG power observed during slow wave sleep

2. ABBREVIATIONS

ASL	Arterial spin labelling
CI	Chief Investigator
CBF	Cerebral blood flow
CRF	Case Report Form
CTRG	Clinical Trials & Research Governance, University of Oxford
EAB	Ethical Advisory Board (LUMINOUS consortium)
EEG	Electroencephalography
FMRI	Functional magnetic resonance imaging
FMRIB	Centre for Functional MRI of the Brain
GCP	Good Clinical Practice
GP	General Practitioner
HRA	Health Research Authority
ICF	Informed Consent Form
LOBR	Loss of behavioural responsiveness
MEG	Magnetoencephalography
MRS	Magnetic Resonance Spectroscopy
NHS	National Health Service
NRES	National Research Ethics Service
OUH	Oxford University Hospitals
PI	Principal Investigator
PIL	Participant/ Patient Information Leaflet
R&D	NHS Trust R&D Department
REC	Research Ethics Committee
SOP	Standard Operating Procedure
SWA	Slow wave activity
SWAS	Slow wave activity saturation
VTE	Venous thromboembolic event (VTE)
WIN	Wellcome Centre for Integrative Neuroimaging

3. BACKGROUND AND RATIONALE

3.1. Background

General anaesthesia typically comprises a triad of hypnosis, analgesia and muscle relaxation. Hypnotic agents, such as intravenous propofol or volatile anaesthetic agents, are delivered to prevent the experience of surgery. However, despite the worldwide daily administration of general anaesthesia, anaesthetists still have no robust and reliable way of knowing when an individual experiencing general anesthesia stops perceiving the outside world. Consequently, many anaesthetists increase the population-based anaesthetic dose to provide a safety margin that ensures the patient is unaware during surgery. Despite this some patients still experience intraoperative awareness, which can have distressing long-term psychological consequences [1]. Potentially more importantly, there is an increasingly recognised risk of adverse short and long-term post-operative outcomes associated with over-anaesthesia, particularly in elderly and vulnerable patients [2–4].

Various currently available depth of anaesthesia (DOA) monitors generate indexes to indicate the level of consciousness. These are typically derived from non-specific, population-based electroencephalographic (EEG) measures of brain activity, and have have limited neurobiological supporting evidence. Importantly, the existing commercial solutions do not relate directly to the specific neurophysiological changes in brain activity induced by anaesthetic drugs (or adjunct anaesthetic agents). The index recommended ranges are typically calibrated using population data rather than related to individual responses. Consequently, currently available depth of anaesthesia do not reliably distinguish responsiveness [5] and are significantly influenced by the anaesthetic regime used [6]. Furthermore, recent systematic reviews have concluded that these DOA monitors demonstrate limited efficacy in reducing accidental awareness compared with standard clinical monitoring [7,8]. Despite this, recent National Institute for Health and Care Excellence (NICE) guidance still recommends that DOA monitors are used for patients at higher risk of adverse outcomes and those receiving total intravenous anaesthesia [8]. These issues highlight that there is a clear need for an individualised brain-based DOA monitor that allows the delivery of the exact anaesthetic dose to achieve perception loss within that individual.

Our neuroscientific understanding of the consciousness has significantly advanced in recent years but still poses a major challenge. Delivery of anaesthesia during EEG and functional neuroimaging offers a powerful tool to reversibly modulate the level of consciousness within an individual and investigate the neurobiology underlying consciousness. Early brain imaging studies on altered states of consciousness identified a specific reduction in thalamic activity and metabolism during the anaesthesia-induced transition to loss of consciousness. This led to the concept of a "thalamocortical switch”, where it was proposed that suppression of the thalamus acts as a barrier to further processing within cortical regions [9–12]. We have proposed an alternative view that these observed reductions in metabolism and activity could be secondary to functional thalamic deafferentation as a result of reduced inputs from brainstem or cortex. Thus, thalamic deactivation may be a consequence of impaired consciousness rather than the cause. More recent data from multiple neuroimaging techniques has produced largely conflicting evidence with regards to the degree of network connectivity disruption in the large-scale thalamocortical and cortico-cortical networks observed during anaesthesia [13–20]. Further in-depth investigation is clearly needed to clarify the role of thalamocortical processing under anaesthesia and the mechanisms underlying its disruption.

3.2. Scientific Rationale

Over five years ago, we performed two anaesthesia neuroimaging experiments in healthy volunteers at the Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB), now part of the Wellcome Centre for Integrative Neuroimaging (WIN). These experiments aimed to identify individualised changes in brain activity associated with loss of consciousness during general anaesthesia. We obtained EEG and simultaneous EEG-FMRI data during an induction of and emergence from deep propofol sedation. By slowing the induction down considerably (over 45 minutes) while presenting noxious and auditory stimuli requiring motor responses, we were able to identify the precise moment when individual subjects became unresponsive and track the subsequent changes in brain activity associated with their loss of consciousness [21,22].

Using EEG spectral analysis, we observed dramatic changes in 0.5-1.5Hz slow wave activity associated with this transition to loss of consciousness. We also found that after loss of behavioural responsiveness, each individual's relative slow wave activity (SWA) (0.5-1.5Hz) - the characteristic brain wave seen in sleep and studies of anaesthesia [23] - rose to saturation, but then remained constant despite increasing propofol concentrations [22]. The equivalent FMRI data showed that when subjects become unresponsive (but pre-saturation), there was sustained brain activity in the modality-specific thalamocortical networks associated with the processing of sensory stimuli [21]. However, at concentrations in excess of slow wave activity saturation (SWAS), a completely different network was active that included the precuneus cortex. This brain region in particular has been recently described as a posterior cortical ‘hot zone’ that is the main anatomical location of the neural correlates of consciousness [24].

Furthermore, we found that an individual’s peak slow wave activity correlated with the grey matter volume of their pre-frontal cortex. This suggests a direct neurobiological relationship between SWAS (i.e. our later electrophysiologically defined transition within the unresponsive period) and the number of bistable oscillating cortical neurons, which are the underpinning basis of cortically measured slow waves [25]. We hypothesized that SWAS is an important individualized EEG end-point that is a manifestation of perception loss to incoming stimuli, and therefore reflects thalamocortical isolation from the external world. As such, SWAS provides a potential target for the titration of anaesthesia to achieve optimum dosing for perception loss in an individual patient. This could further reduce the risk of mortality and morbidity from over- and under-anaesthesia, particularly in elderly and vulnerable patients [26–28].

We have expanded our findings to the less well-controlled, clinical environment during surgical anaesthesia [29]. We have demonstrated that SWAS can be observed during surgical anaesthesia. Specifically, we characterised the slow wave activity dose-response relationship on an individual basis using EEG data from our previous experimental study and three other clinical studies [22,30–32]. Using retrospective post-hoc model fitting, we identified a plateau in the SWA dose-response (indicative of SWAS) in 92% of a total of 393 EEG datasets, with the majority of failed fits due to muscle or movement artifacts in the EEG. We showed that SWAS occurs on induction and emergence for both intravenous and volatile anaesthetic agents. We also found that SWAS occurs in the presence of anaesthetic co-induction agents, such as opioid analgesia and neuromuscular blockades. This formed an important first stage in the clinical translation of SWAS for depth of anaesthesia monitoring.

3.3. Outline of main research questions

This protocol outlines the next stage in the development of SWAS as a marker of depth of anaesthesia. The research has two major aims that will be achieved through experiments in two study populations: patients undergoing surgery and healthy volunteers. Firstly, it aims to demonstrate that it is possible to identify SWAS on an individual basis in real-time using EEG feedback. Secondly, it will confirm that individuals held at SWAS have no conscious content and experience perception loss through a disruption in thalamocortical connectivity.

We have recently developed a mathematical model that will enable us to identify SWAS in individuals in real-time, and that will enable delivery anaesthesia appropriately to achieve this end-point. The SWAS prediction model dynamically monitors the change in the brain's slow wave activity with varying anaesthetic drug concentrations, and uses Bayesian statistics to predict when SWAS has been achieved for that individual. Bayesian models are particularly useful as they update the probability of a hypothesis as more evidence or information becomes available. Our Bayesian model has been developed specially to detect SWAS but similar Bayesian prediction models are used in many other areas (e.g. climate change, engineering control systems, finance, etc.). By applying this prediction model retrospectively to our previously acquired EEG datasets, we have already gained much knowledge about the inter-and intra-individual variability of these dynamic slow wave activity changes, and their relationship with the SWAS end-point. This information serves to inform the Bayesian prior distribution so that we are better able to predict whether a given individual has achieved SWAS.

A limitation in the application of our real-time model to previous data is that these were largely collected in observational studies where the clinical anaesthetic concentrations delivered were often far in excess of SWAS. Therefore, our first study aims to apply the SWAS prediction model to patients receiving anaesthesia to optimise the dosing of anaesthesia in real-time to achieve and maintain an individual at SWAS (Study 1). This study will be performed in patients during induction of anaesthesia before surgery takes place. We will alter how the patients are 'put to sleep' by delivering the hypnotic component of anaesthesia according to each patient's brain activity recorded until we achieve SWAS. We will apply the SWAS prediction model to changes in brain activity elicited by two commonly used hypnotic agents, intravenous propofol and inhalational sevoflurane. In the first instance, this will require slowing the induction process down (i.e. the reducing the rate of drug delivery) so that we can optimise the SWAS prediction model for real-time operation. We will aim to optimise the real-time operation of the model for each hypnotic agent until we can achieve SWAS within 15 minutes, similar to the time required for a standard clinical induction of anaesthesia. After we have identified and maintained SWAS for a short time in each patient, the clinical team will take over the care of the patient and their clinical care will continue as normal. The clinical team will use standard clinical procedures to optimise the level of anaesthesia for the patient, ensuring that all patients are sufficiently anaesthetised prior to surgery.

In our second study, we will then apply the SWAS prediction model and use magnetic resonance imaging techniques to further explore the brain mechanisms underlying SWAS in healthy volunteers (Study 2). We will use simultaneous EEG and MRI data recordings during inhalational sevoflurane anaesthesia to explore how the thalamus changes its connectivity with the cortex when at SWAS compared with wakefulness. During these experiments, we will also confirm that individuals held at the SWAS endpoint are unresponsive to painful and auditory stimuli. These imaging experiments aim to validate that SWAS is a useful endpoint that truly indicates perception loss under general anaesthesia.

Finally, although anaesthesia and sleep are fundamentally different states, they do share some common mechanisms and can influence each other [23,33,34]. By using a within-subject design, we will explore the commonality and bidirectional influence of these mechanisms further by using EEG sleep recordings prior to anaesthesia delivery during neuroimaging.

3.4. Potential risks and benefits

There are no immediate benefits to the individuals taking part in the planned research studies, apart from the knowledge that they are contributing to scientific research that we envisage will advance future medical care.

By performing this scientific research, we will significantly enhance our systems neuroscience level understanding of anaesthetic mechanisms and consciousness. Specifically, a better understanding of the neural mechanisms underlying SWAS will help us develop our individualised brain based measure of depth of anaesthesia. In the longer term, we hope that using SWAS to guide the optimum anaesthetic dose to achieve perception loss within an individual will help prevent both under- and over-anaesthesia during surgery.

This optimization of anaesthetic dosing has cost implications for all sectors of the health care system. Even a small reduction in the quantity of anaesthetic drugs required for the 2.9 million anaesthetics delivered annually in the UK would have significant cost savings. The anaesthetic community would also welcome any reduction in the unpredictability of patient responses that ultimately allows a more consistent anaesthetic delivery with improved patient outcomes.

Excessively deep anaesthesia has been linked to an increased risk of adverse outcomes such as death, stroke, heart attack, delirium and cognitive dysfunction. Patients at higher risk of these adverse outcomes will benefit most from this optimization of anaesthetic dosing. These include the elderly and people with poor cardiovascular function and high body mass indices. With an aging population, post-operative cognitive impairment outcome measures have become increasingly important in the risk-benefit decision of whether to operate or not. Improved recovery due to optimal dosing will reduce the time to discharge, enabling patients to return home more quickly.

We have covered the ethical considerations of the study procedures in section 12.7. We expect that primary concern of the ethics committee will be the delivery of anaesthesia to healthy volunteers, as 3T MRI scanning and simultaneous EEG recording are safe, non-invasive imaging techniques when the necessary safety precautions are followed.

Our group and the WIN have over 18 years of experience of performing EEG, pharmacological MRI and combined EEG-FMRI in both healthy and vulnerable adults. The procedures for delivery of anaesthesia to healthy volunteers will be largely reproduced from our previous LREC approved studies using the intravenous anaesthetic agent propofol (LREC: 09/H0605/57). We will follow the Anaesthetic Association of Great Britain and Ireland's (AAGBI) guidelines for day case surgery (https://www.rcoa.ac.uk/system/files/GPAS-2016-06-DAYSURGERY.pdf) for the experiments involving anaesthesia delivery. Anaesthesia will be delivered by a consultant-grade anaesthetist, who will stop the study at any time if concerned about the safety of the participant.

Individuals who undergo a surgical procedure with a total anaesthetic and surgical time of more than 90 minutes, or 60 minutes if the surgery involves the pelvis or lower limb, have an increased risk of

experiencing a venous thrombosis event (VTE) [35]. There is a minor concern that increases in anaesthetic time for patients in Study 1, and the delivery of anaesthesia to the healthy volunteers, will increase the risk of a VTE due to prolonged immobilisation. We will reduce the risk of a VTE occurrence during the studies by recruiting healthy volunteers and patients who have a low risk of developing a VTE. We will also use standard clinical prophylactic measures (e.g. anti-embolism compression stockings) to mitigate any increased risk due to immobilisation during anaesthesia.

4. OBJECTIVES AND OUTCOME MEASURES

Objectives	**Outcome Measures**	**Evaluation time-point**
Primary Objective 1. Assess resting and functional thalamocortical connectivity at loss of behavioural responsiveness (LOBR) and slow wave activity saturation (SWAS)	Changes in connectivity of sub-regions of thalamus with the cortex at LOBR and SWAS compared with the baseline (no anaesthesia) condition during resting and task-based functional imaging scans.	Study 2 end
Secondary Objectives 1. Demonstrate that it is possible to titrate anaesthesia to SWAS on individual basis using real-time EEG feedback	Achieve stable SWAS endpoint on an individual basis using propofol and sevoflurane anaesthesia within 15 minutes	End of Study 1 and 2
2. Demonstrate that individuals held at SWAS are unresponsive to pain and have no conscious content as assessed by the isolated forearm test.	a) Significant reduction of FMRI thalamacortical brain activity in response to pain at SWAS compared with loss of responsiveness b) No behavioural response (as assessed by hand open/close) to verbal request when held at SWAS	Study 2 end
3. Explore common mechanisms and influence of sleep on anaesthesia	Within-subject correlation of EEG power at SWAS under anaesthesia and maximum EEG power observed during slow wave sleep	Study 2 end

5. STUDY DESIGN

5.1. Overview

The research aims will be achieved through experiments in two study populations: patients undergoing surgery and healthy volunteers. Study 1 will demonstrate that it is feasible to titrate anaesthesia to achieve (and maintain) SWAS in an individual patient using real-time EEG feedback. Study 2 is a healthy volunteer neuroimaging study that will identify changes in thalamocortical connectivity at SWAS and confirm that SWAS indicates perception loss.

The patient study (Study 1) will commence prior to the healthy volunteer study (Study 2), and will continue alongside to iteratively improve anaesthetic delivery using real-time EEG feedback. Both studies will recruit up to 30 participants.

5.2. Patient study (Study 1)

We will perform this study in patients who are having general anaesthesia for elective plastic surgery in the Oxford University Hospitals (OUH) NHS Foundation Trust. The schedule of procedures is included in 0. In summary, the study will be conducted as follows:

Recruitment/eligibility assessment

1) Advertisement and recruitment for the study by clinical staff on participating elective surgical lists
2) Telephone / face-to-face explanation of the study and assessment of eligibility by research team (10-15 minutes)

Day of surgery

3) Allocation of patient to receive either inhalational sevoflurane or intravenous propofol or anaesthetic induction to SWAS
4) Informed consent on the day of surgery or at the earlier pre-operative assessment appointment (10-15 minutes)
5) Set-up of EEG monitoring on the patient before anaesthesia begins (20-30 minutes)
6) Questionnaire booklet completed by patient (15-20 minutes) - may be concurrent with EEG set-up
7) Delivery of anaesthesia to achieve (and maintain) SWAS by study anaesthetist (ranging from 15 minutes -1 hour)
8) Handover to clinical anaesthetist
9) Routine clinical anaesthesia and surgery with ongoing EEG data collection

Acute post-operative

10) Normal patient recovery following surgery with ongoing EEG data collection
11) EEG monitoring removed when patient is awake and responsive (5 minutes)
12) Patient interviewed about intraoperative experiences (5 minutes)

Post-operative follow-up

13) Patient sent a study satisfaction questionnaire 3-4 weeks post operatively (5 minutes)

14) Additional telephone interview for patients with intraoperative experiences (5 minutes)

Refinements to the SWAS prediction model will be made after each patient to optimise the model and decrease the anaesthetic time required to achieve SWAS. In the initial stages, we expect the session to take up to 1 hour extra in the anaesthetic room, with an increased anaesthetic drug delivery time of approximately 30 minutes per person.

The study end-point is defined as the ability to reliably achieve SWAS on induction within 15 minutes using either propofol or sevoflurane anaesthesia (i.e. comparable with a normal clinical anaesthetic induction).

5.3. Healthy volunteer study (Study 2)

An overview of the schedule of procedures for the healthy volunteer study is presented in Appendix B and Appendix C. Potential volunteers will be invited to attend a medical screening appointment to check their suitability for participation (Visit 1). Suitable and consented healthy volunteers will then experience two 3T MRI sessions at the WIN (Visits 2 and 3). The first will be a baseline session and the second session will involve the delivery of sevoflurane anaesthesia during EEG-MRI data acquisition. An overnight EEG recording of the participants' sleep will take place between these two MRI sessions. These visits are outlined further below:

Visit 1 - medical screening (30-45 minutes)

This take place in the Nuffield Department of Clinical Neuroscience or John Radcliffe Hospital and will include:

- in-depth explanation of the study procedures
- full informed consent
- anaesthetic assessment by a post FRCA or equivalent anaesthetist, and
- additional consultation with the MR radiographers (if required).

MRI Visit 2 (2 hours)

The first MRI session will be carried out in the early evening and will involve a non-invasive recording of anatomical, neurochemical and cerebral blood flow (CBF) measurements (approximately 1-1.5 hours).

Volunteers will also complete a set of validated questionnaires relating to their general level of anxiety, sleep quality, and personality.

The ambulatory sleep EEG kit will be applied after scanning (30 minutes) and volunteers will then return home in a taxi to experience a night of sleep recording in their own home. This session will take approximately 2 hours to complete in total.

MRI Visit 3 (4-5 hours)

The volunteers will return early the following morning, having fasted overnight. Prior to the second MRI session, they will have the sleep EEG kit removed (10 minutes) and the MRI-compatible EEG kit applied (30 minutes). A short EEG recording of resting brain activity will be acquired outside of the scanner to check data quality. Volunteers will also complete further questionnaires relating to their level of state anxiety, and sleep quality the previous night (10 minutes). This will take around 60 minutes in total.

Volunteers will then be positioned in the scanner. Simultaneous EEG-MRI data will be acquired at baseline and during sevoflurane anaesthesia delivery. Anaesthesia will be delivered to 1) loss of behavioural responsiveness to auditory stimuli, and 2) the SWAS EEG endpoint as determined by the SWAS prediction model. We will perform the same neurochemical and cerebral blood flow measures as acquired in the first MRI session on the previous day. Additionally, we will measure the individuals' brain activity at rest and in response to pain and word stimuli using FMRI.

Volunteers will then be removed from the scanner bore. They will be allowed to emerge naturally from anaesthesia lying on the scanner table in the presence of the anaesthetist while EEG data acquisition continues. When the volunteers are awake and oriented they will transfer to an adjoining room and be supervised until they recover. Due to individual variability in the susceptibility to anaesthesia, the time to complete this session will vary across volunteers. We expect the whole session to last between 4-5 hours in total, with delivery of sevoflurane anaesthesia for approximately 2-2.5 hours.

6. PARTICIPANT IDENTIFICATION

6.1. Study Participants

We will recruit both elective plastic surgery patients (Study 1) and healthy volunteers (Study 2).

6.2. Inclusion Criteria

All study participants will be required to meet the inclusion criteria outlined below:

1. Willing and able to give informed consent
2. Male or female
3. Aged 18-60 years
4. American Society of Anesthesiology (ASA) score of 1 or 2
5. English speaking

The age range and ASA status of the study populations have been chosen to select individuals in general good health with similar susceptibilities to anaesthetic drugs. In particular, targeting a younger population will reduce the influence of age on anaesthetic dose requirement [36] and brain structure/function [37]. In the case of healthy volunteer study, it is also ethically questionable whether the older population should receive anaesthetics that are not clinically required.

6.3. Exclusion Criteria

The general exclusion criteria apply to both studies, whereas the surgical and MRI exclusion criteria only apply to Studies 1 and 2 respectively. With a view to recruiting individuals with a low VTE risk, we have excluded obese individuals (body mass index >30 kg/m^2), those using hormone replacement therapy and those with a personal history or first-degree relative with a history of VTE according to the NICE guidelines [35]. The contraceptive pill also increases the risk of a blood clot developing. However, the baseline risk is small with increased risk of around six extra cases of blood clot for every 10,000 women prescribed the contraceptive pill. Given the age range of the study population, we are concerned that to exclude women on contraceptive medication would potentially bias our study cohort. Therefore, we have decided not excluded women using oestrogen-containing contraceptives.

6.3.1. General exclusion criteria

1. Smoker (tobacco or electronic cigarettes)
2. Illicit drug use
3. High alcohol intake (>14 units/week)
4. Pregnancy
5. Prescription medication that could influence the EEG or interact with anaesthesia
6. Personal or familial history of allergies and/or adverse reactions to anaesthesia
7. History or clinical signs of potential susceptibility to airway obstruction, and known difficult airway
8. Personal or familial history of epilepsy, other neurological disorder or psychiatric disorder
9. History of psychological pathology, chronic pain, migraines or dementia
10. Increased risk of venous thrombo-embolic event (VTE), defined by
    a. Obesity (body mass index >30 kg/m^2)
    b. Personal history or first-degree relative with a history of VTE
    c. Use of hormone replacement therapy
11. Vulnerable group status:
    a. Drug, alcohol or substance abuse issues
    b. Learning difficulties
    c. Difficulty reading or speaking English
    d. Homeless, asylum seekers/refugees or those with no recourse to public funds
    e. In contact with prison or probation services

6.3.2. Surgical exclusion criteria (Study 1)

1. Surgery involving the head or neck, or the prone position
2. Patients involved in litigation cases
3. Other surgical reason at the research or clinical team's discretion

6.3.3. MRI exclusion criteria (Study 2)

1. Left-handed
2. Claustrophobia
3. Certain metallic implants
4. Metallic injury to eye
5. Tattoos (depending on location)
6. Individuals with facial hair and beards

7. STUDY PROCEDURES

7.1. Patient Study

A summary of the schedule of procedures for the patient study is provided in 0.

7.1.1. Recruitment

We will initially recruit patients under the care of Dr Jane Quinlan (OUH consultant anaesthetist) and her anaesthetic colleagues, and Miss Nicola Petrie, Mr David Coleman, Mr Peter Kalu and Mr Jeremy Birch (OUH consultant plastic surgeons). The surgical, anaesthetic and pre-operative assessment teams will identify patients who meet the study inclusion criteria. The NHS clinical teams will perform an initial screening of patients on the basis of the primary inclusion criteria, the surgical exclusion criteria and vulnerable group status. Additionally, we will seek to recruit patients who be ordered 2nd or later on a surgical list so as to reduce the potential for introducing timing delays.

Suitable patients will be invited to participate by the clinical team at either their i) outpatient or ii) pre-operative assessment appointment. They will be given a brief explanation of the study, an invitation letter and a patient information sheet (PIS) to take home. It will be clearly explained to the patient that there is no obligation to participate and that non-participation will not influence their future medical care.

After the initial explanation of the study, patients will be asked if they are happy to be contacted by the research team. The research team will also be available at the pre-operative assessment appointment to answer any questions about the study, and check eligibility should the patient wish.

7.1.2. Screening and Eligibility Assessment

The research team will contact interested patients by telephone or email to discuss the study further. At this time, they will confirm that the patient understands what is involved in the study. Should a patient still express an interest in participating, the research team will carry out a full eligibility assessment according to the study's inclusion and exclusion criteria.

It will be explained to the screened participants that they will be contacted again to confirm their date of surgery. This will also serve to check the patient is still happy to take part closer to the time of their operation. The potential participants will also be given contact details for the research team should they have any questions.

7.1.3. Informed Consent

Written and verbal versions of the Participant Information and Informed Consent will be presented to the participants detailing no less than: the exact nature of the study; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, without affecting their legal rights, and with no obligation to give the reason for withdrawal. The participant will be allowed as much time as they wish to consider the information, and have the opportunity to question the Investigator, their GP or other

independent parties to decide whether they will participate in the study. The participant must provide written Informed Consent form before any study specific procedures are performed.

Written Informed Consent will be obtained by means of participant dated signature and dated signature of the person who presented and obtained the Informed Consent. The person who obtains the consent will be suitably qualified and experienced, and have been authorised to do so by the Chief Investigator. A copy of the signed Informed Consent form will be given to the participant. The original signed form will be retained at the study site and a third copy will be put in the patient's notes.

Full informed consent will take place either on the morning of surgery, or at the earlier pre-operative assessment clinic if the patient is happy to proceed at this time.

7.1.4. Study Visit

Patients will attend the Oxford University Hospitals Trust on the day of surgery as normal. The NHS standard procedures to prepare the patient for surgery will be carried out. In addition, both the clinical anaesthetist and the study anaesthetist will meet with the patient to discuss the anaesthetic. Once the clinical anaesthetist is happy with the standard preoperative check in, informed consent will be obtained by study personnel (if required). The additional study procedures will then be performed:

7.1.4.1. Electroencephalography

Consented patients will have an EEG cap or an arrangement of single electrodes applied to the scalp while waiting for surgery. We will use a CE-marked EEG system (PortiEEG, TMSI, Netherlands) that has previously been used in the operating room environment in a small pilot study carried out at Milton Keynes General Hospital (LREC ref: 14/SC/0145, IRAS ref: 138286). Depending on room availability and expected surgical list timings on the day, we will either apply the EEG cap or electrodes in a separate room or in the anaesthetic room where the patient will be prepared for surgery.

Sensor placement and preparation of the scalp EEG requires about 20-30 minutes. The procedure typically involves the use of a snug fitting cap made of an elasticated cloth material and containing tin or silver/silver chloride electrodes, and establishing electrical contact between the scalp and the electrodes by means of an electrolyte gel or water solution. In order to achieve a low-impedance connection, it is often necessary to prepare the area of the scalp under the sensor by cleaning it with rubbing alcohol and rubbing an abrasive substance using a cotton swab or by scratching the surface of the scalp with a blunt wooden stick. The procedure does not ordinarily cause pain or harm to the participant.

7.1.4.2. Questionnaires

The patient will be asked to fill out a short questionnaire booklet either while they wait for surgery or when they are having the EEG cap/electrodes applied. These questionnaires ask the patient about their pre-operative sleep and anxiety levels. Pre-operative anxiety and pain sensitivity have been shown to be independent predictors of propofol and sevoflurane requirements in general anaesthesia using BIS [38] and we would like to investigate the same relationship with the SWAS biomarker. Poor sleep and anxiety are also highly correlated and exploring the commonalities of sleep and anaesthesia is one of the secondary study objectives.

We have also included a questionnaire to evaluate a personality trait called absorption, which is a sub-scale of the Multi-dimensional personality questionnaire (http://www.upress.umn.edu/test-division/mpq). It has been suggested that absorption measures the readiness of an individual to restructure their representation of self and its boundaries [39], and has been found to correlate well with hypnotic susceptibility [40]. Our previous work suggests that individuals experience a loss of selfhood when they become unresponsiveness under anaesthesia [21]. In the next study, we would like to investigate whether the degree of thalamocortical connectivity changes that occur at loss of responsiveness reflect this ability for individuals to disengage from their representation of self and their external environment.

Volunteers are more open to new experiences as evidenced by their study participation. They therefore are likely to score higher on the correlated absorption scale. We hypothesise that individuals who have higher absorption scores will require less anaesthesia to achieve loss of responsiveness. We would like to include this questionnaire in the patient study, as there is likely to be more variability in absorption scores due to the non-voluntary nature of the surgical anaesthesia.

This patient booklet therefore includes the following validated questionnaires:

- State and trait anxiety index (STAI) -Trait component [41]
- Amsterdam Preoperative Anxiety and Information Scale [42]
- Pittsburgh sleep quality index (PSQI) [43]
- Tellegen absorption scale (TAS) [39]

We have also asked an additional question about the quality of their previous night's sleep. The patients will be asked to rate the question "I slept well last night" on a scale of 1 (not at all) to 5 (extremely).

7.1.4.3. Anaesthetic delivery to SWAS

Patients will be administered either a propofol and/or sevoflurane anaesthetic induction to the SWAS endpoint by an appropriately qualified anaesthetist, such consultant anaesthetists Dr Richard Rogers and Dr Lara Prisco. These procedures will take place in the anaesthetic room, and the recommended standards of patient monitoring and trained anaesthetic assistance will be met for each patient as per standard clinical practice (Royal College of Anaesthetists' Guidelines for the Provision of Anaesthetic Services (GPAS) 2016, http://www.rcoa.ac.uk/node/21849). We will also have an Anaesthetic nurse or Operating Department Practitioner present during this time.

We will use the SWAS prediction model applied to real-time EEG brain signals recorded from the scalp to titrate anaesthesia to achieve SWAS. The patient will be maintained at this end-point for 10 minutes. The patient's airway will be managed as, and when, is clinically appropriate through the insertion of a laryngeal mask or intubation as is standard clinical practice. If required, after the propofol inductions, conversion to a volatile anaesthetic agent may be used for maintenance (according to the clinical anaesthetist's standard practice). In these cases, we will use EEG feedback and the SWAS prediction model again to titrate the sevoflurane to the SWAS endpoint.

Each time the individual is held at the SWAS endpoint they will be asked "[PATIENT'S NAME], squeeze my hand" to assess for any conscious content [44].

7.1.4.4. Allocation of anaesthetic agent

Both propofol and sevoflurane hypnotic agents are used for clinical induction of anaesthesia in adults. Both anaesthetic agents will be used but will not be randomised. We perform the anaesthetic inductions using inhalational sevoflurane first as this agent will be used during the healthy volunteer study. After we have achieved the study end-point with sevoflurane, we will then administer propofol inductions in the remaining participants until we achieve the study end-point with this hypnotic agent, which is most commonly used for induction of anaesthesia in adults.

Should an eligible patient not tolerate the inhalational sevoflurane agent well during induction, they may be give intravenous propofol induction to SWAS rather than lose that patient from the study. Similarly, if a needle-phobic patient was interested in participating, they may be given inhalational sevoflurane instead of intravenous propofol anaesthesia induction.

7.1.4.5. Additional study procedures

When appropriate, the care of the patient will be transferred to the NHS consultant anaesthetist on the surgical list. All other clinical procedures including the delivery of anaesthesia will then continue as normal. Perioperative data will be collected from the anaesthetic chart to capture the procedures performed by the NHS anaesthetist. Additionally, EEG monitoring will continue throughout surgery and emergence from anaesthesia but will not interfere with clinical care. Collection of physiological data and recording of drug concentrations will also be performed throughout.

Specifically, the research team will record the following during anaesthesia delivery:

- time and dose of the various intravenous hypnotic and analgesic drugs
- time of loss and recovery of behavioural response
- time of insertion and removal of airway devices
- time of first surgical incision
- heart rate, blood pressure, end-tidal carbon dioxide concentrations, end-tidal anaesthetic concentrations, oxygen saturation levels of the patient.

Any major emergencies during surgery will be dealt by the clinical team as per OUH NHS Foundation Trust guidelines.

7.1.4.6. Post-operative follow-up

When participants are awake, they will be administered the Nursing Delirium Screening Scale [48]. The EEG cap or electrode removal will take place when the patient is fully awake and responsive, and the clinical anaesthetist believes that the patient has recovered sufficiently from the anaesthetic. They will be asked the brief validated Brice Awareness questionnaire by the research team to assess for any intraoperative experiences under anaesthesia [45]. If the patient reports any intraoperative experiences at this time, they will be contacted again by telephone at a later date (3-4 weeks) to review their account, as per the questionnaire guidelines. Audio recordings of the patient's responses to the Brice questionnaire may be performed to allow transcription and interpretation at a later date.

Participants will also be sent a feedback sheet approximately three to four weeks after study completion. It will be made clear that its completion is entirely voluntary but if returned will be used to guide patient involvement in future studies. We will also ask if they patients wish to be invited to future Patient-Public

Involvement (PPI) initiatives. For participants who have expressed a wish to be contacted about the success of the study, we will write to them detailing the study outcomes at its conclusion.

7.2. Healthy volunteer study

A summary of the schedule of procedures for the healthy volunteer study is provided in Appendix B.

7.2.1. Recruitment

Participants will be recruited by word of mouth, social media, emails to departmental and college mailing lists, and posters located in University Departments, and on the Oxford University Hospitals NHS Trust sites. Contact may also be made with previous study participants, who have indicated that they are interested in future studies and are happy to receive information about these. Participants may also be recruited from online study recruitment websites such as TrialSpark (https://www.trialspark.com), which is currently used by our group for other ethically approved studies. Interested volunteers will receive a participant information sheet (PIS) that provides full details of the study. Subsequent inclusion in the study will be subject to appropriate and timely scheduling of the medical screening and MR sessions.

7.2.2. Eligibility Assessment

Volunteers will be screened carefully with regard to the risks associated with undergoing anaesthesia, EEG and MRI. Firstly, the research team will assess the suitability of potential volunteers against the study inclusion/exclusion criteria over the telephone using the Volunteer Health Checklist. Volunteers will also be asked to complete an MRI screening form at this stage (and return by post or email) to identify any concerns relating to their safety in the MR environment. These will be reviewed in collaboration with the radiographers at the WIN to assess any safety concerns. We will follow WIN's Safe Surgery/Implant Policy to ensure eligibility and their safe participation. In cases of ambiguity as to whether it is safe to scan a volunteer due to a previous surgical procedure or implant, medical records may be requested if the participant gives permission (in-line with the WIN policy).

We will also ask participants to bring some identification and proof of address with them, as this is University policy for experiments involving sleep recording in the participant's own home.

7.2.3. Medical Screening (Visit 1)

Suitable volunteers will be invited to attend a screening appointment at a mutually convenient time. This visit will involve obtaining informed consent and completion of a full medical and safety screening by a study researcher and a post-FRCA (or equivalent) anaesthetist.

7.2.3.1. Contraindications to MRI

Prior to obtaining informed consent, the study researcher will review the study inclusion/exclusion criteria and completed MR screening form in person with the volunteer. We will follow the WIN Safe Surgery/Implant Policy to ensure eligibility and their safe participation. An additional consultation with the MR radiographers will be sought should any concerns arise as part of this discussion. On some occasions, for example to assess the safety of an implanted device, this may require access to the

subject's medical notes. In these cases, informed consent will be obtained prior to accessing medical notes.

7.2.3.2. Explanation of the study and participant responsibilities

Participants will receive an in-depth explanation of the study procedures before informed consent is obtained. This will involve a demonstration of the equipment and procedures that will be used during the study sessions. For example, we will ask them to briefly try on the EEG cap and the facemask that will be used to deliver the sevoflurane anaesthesia. We may also ask the volunteer to briefly lie on the MR scanner table (outside of the bore) so that we can assess how the facemask fits with the MR head coil.

The responsibilities of the participant as part of the study will be highlighted at this visit. For example, the importance of fasting prior receiving anaesthesia will be explained, and how they will need to be accompanied at home on the evening following the anaesthetic session.

7.2.3.1. Informed Consent

As with the patient study, the participant must personally sign and date the latest approved version of the Informed Consent form before any study specific procedures are performed.

Written and verbal versions of the Participant Information and Informed Consent will be presented to the participants detailing no less than: the exact nature of the study; what it will involve for the participant; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw from the study at any time for any reason without prejudice to future care, without affecting their legal rights, and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as wished to consider the information, and the opportunity to question the Investigator, their GP or other independent parties to decide whether they will participate in the study. Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the Informed Consent. The person who obtains the consent must be suitably qualified and experienced, and have been authorised to do so by the Chief Investigator. A copy of the signed Informed Consent will be given to the participant. The original signed form will be retained in the study site file.

7.2.3.2. Suitability for anaesthesia

We will perform a separate medical assessment to ensure that the volunteer can safely be administered sevoflurane anesthesia in the scanner. This will include:

- Medical history: participants will be asked about any current or past medical problems including adverse reactions to anaesthesia. They will also be asked about current medications, including both prescription and over-the-counter drugs, and pregnancy status. Women who are unsure if they are pregnant will be offered a pregnancy test.
- Demographics: the participants' age, weight and height will be recorded.
- Social history: participants will be asked about their smoking history, alcohol use, illicit drug use and caffeine consumption. They will also be assessed against the vulnerable group criteria.

- Family history: participants will be asked whether any of their first-degree relatives have/had any allergies, adverse reactions to anaesthesia, and psychiatric or neurological illnesses.
- Formal airway assessment: participants will be screened by an anesthetist to exclude individuals with potential for obstruction in the scanner. They will be asked if they have any history of airway obstruction or known difficult airway. They will have a physical examination to assess for clinical signs of potential susceptibility.
- Assessment of venous thromboembolic event risk: a formal assessment will be performed by an anesthetist against the current NICE guidelines [35].

7.2.4. Visit 2 – MRI Baseline assessment

7.2.4.1. MRI data acquisition

On arrival, participants will be asked if they have any relevant changes in their personal circumstances since their medical screening appointment. Participants will fill in another MRI screening form that will be checked by designated and trained scanner operators. They will then get changed into loose fitting comfortable clothing (scrubs) provided by the WIN in a private room. They will be positioned in the 3T Siemens Prisma scanner. Foam padding will be placed around the participant's head to minimise movement. Participants will wear earplugs to attenuate the noise of the scanner. Participants will also view an angled mirror to allow them to see out of the end of the magnet base. At all times, participants will be able to indicate immediately if they wish the scanning to be terminated by squeezing a buzzer placed in the hand. During scans, the participant is visually monitored from the control room.

The scanning session will last approximately 60 minutes, and will consist of several different brain imaging sequences. Specifically, these MRI scans may include:

- structural scan (T1) - high resolution anatomical image of the brain
- diffusion tensor imaging (DTI) - structural white matter pathway measurement
- magnetic resonance spectroscopy (MRS) - neurotransmitter measurement
- arterial spin labeling (ASL) – cerebral blood flow measurement
- resting and functional MRI scans (FMRI)

We will also use physiological monitoring during MR scanning to monitor their heart and respiration rate. These will be used at the analysis stage to clean up the breathing and pulse artifacts in the obtained MR images.

7.2.4.2. Questionnaires

During this visit, they will also fill in a questionnaire booklet containing the following validated questionnaires:

- State and trait anxiety index (STAI) -Trait component [41],
- Pittsburgh sleep quality index (PSQI) [43],
- Tellegen absorption scale (TAS) [39],
- Embodied sense of self scale (ESSS) [46],
- Locus of control (LOC) [47].

In our previous work, we hypothesised that loss of behavioural responsiveness was associated with a loss of selfhood [21]. We have therefore included the Embodied sense of self scale to evaluate how strong each individual's sense of self is and explore how this relates to anaesthetic dose requirement. Furthermore as an exploratory analysis, we have included the Locus of control questionnaire to evaluate if specific disruptions of cortico-cortical or thalamocortical connectivity with increasing levels of anaesthesia is influenced by individuals' need for control.

7.2.4.3. Sleep EEG and overnight requirements

When the MR scanning has completed, the participants will be removed from the scanner and will be able to get changed into their own clothes. They may choose to change into comfortable night clothing or pyjamas at this time. After a short break, they will have an ambulatory sleep EEG kit (e.g. SOMNOscreen plus EEG 32 (SomnoMedics) applied to their scalp. They will then be sent home in a (fare paid) taxi to sleep in their own home. They will be asked to stay at home for the evening, eat a good substantial meal and go to bed at their normal bedtime or whenever they feel tired. They will also be asked to refrain from eating or drinking for six hours before receiving anaesthesia the next day. They will be allowed still water for up to 2 hours before receiving the anaesthesia.

It will be made clear that if they have any food or drink the next morning that the experiment may be cancelled. They will also be reminded that they may not drive, use machinery, drink alcohol or engage in sporting activities on the evening after receiving anaesthesia.

7.2.5. Visit 3 – Sevoflurane anaesthesia delivery during EEG-MRI

7.2.5.1. Preparation, questionnaires and MR screening

The volunteers will return via taxi early the following morning and have the sleep EEG kit removed. They will be asked to confirm that they have not eaten during the preceding six hours.

Participants will get changed in a private room into the WIN's loose fitting clothing before having the MRI-compatible 32 channel EEG cap (BrainCap MR, Easycap GmbH) applied. They will also apply compression stockings to help prevent blood clots due to prolonged immobilisation.

Participants will fill in another MRI screening form that will be checked by the trained scanner operators. Volunteers will also complete questionnaires relating to their level of state anxiety, and sleep quality the previous night. We will use the following questionnaires:

- State and trait anxiety index (STAI) - State component [41], and the
- Pittsburgh sleep quality index (PSQI) – adapted for the previous night's sleep.

7.2.5.2. Delivery of anaesthesia

Volunteers will then be positioned in the scanner as described in Visit 2. They will have a facemask fitted that will be used to deliver inhalational sevoflurane anaesthesia. The anaesthesia will be delivered by/under the supervision of a consultant-grade anaesthetist, who will stop the study at any time if concerned for the safety of the participant. The expected levels of anaesthesia to be used represent a minor risk to the volunteer's airway maintenance. We will additionally deliver oxygen (or air) to the participants to prevent hypoxia. We will follow the Anaesthetic Association of Great Britain and Ireland's

(AAGBI) guidelines for day case surgery. Volunteers will be subjected to the same physiological monitoring as if they were experiencing day case surgery. In addition to monitoring of their heart and respiration rate during scanning (see Visit 2), we will also record their ECG, blood pressure, oxygen saturation levels, end-tidal CO_2, and end-tidal sevoflurane concentration levels. Volunteers will have an intravenous cannula inserted prior to scanning for medication delivery in the case of emergency.

7.2.5.3. MRI-EEG data acquisition

We will collect EEG data using the MR compatible EEG amplifier system (MRplus, BrainVision GmbH). A short recording of resting EEG brain activity will be acquired outside of the scanner to check data quality before proceeding. Simultaneous EEG-MRI data will be acquired at baseline and during sevoflurane anaesthesia delivery. Firstly, there will be a baseline EEG-MRI data acquisition prior to anaesthesia delivery (40 minutes). Sevoflurane anaesthesia will then be delivered slowly during EEG-FMRI data acquisition until the volunteers stop responding to auditory stimulation delivered via headphones. Due to differences in individual susceptibility to anaesthesia, we expect this time period to variable and take between 5-15 minutes (Step 2). The subjects will be then held at this level of anaesthesia (i.e. loss of behavioural responsiveness) and the MRI protocol used at BASELINE will be repeated (Step 3 - 40 minutes at LOBR). We will apply painful and auditory stimuli and perform the modified isolated forearm test at this time to fully assess the participants' responsiveness (see below).

We will then use the SWAS prediction model to titrate anaesthesia to achieve and maintain the SWAS end-point using real-time feedback of the individual's EEG (as in Study 1). Again, due to individual susceptibility to anaesthesia, this time required to achieve SWAS will be variable across volunteers but having optimised this aspect in the previous study in patients we envisage this to take around 10-15 minutes (Step 4). We also hope to collect resting FMRI data whilst the anaesthesia is being titrated to SWAS, if this does not prove to be too technically challenging. Finally, the same MRI protocol used at BASELINE and LOBR will be repeated while the individuals are maintained at SWAS (Step 5 - 40 minutes at SWAS). Again, we will apply painful and auditory stimuli and perform the modified isolated forearm test at this time to fully assess the participants' responsiveness.

In summary, the scanning protocol is formed of five parts with the following MRI data acquisitions (see Appendix C):

1. BASELINE (prior to dosing): MRS, ASL, resting and task FMRI (pain + words)
2. Delivery of auditory stimuli until LOBR (during task FMRI)
3. LOBR: MRS, ASL, resting and task FMRI (pain + words)
4. Real-time titration of anaesthesia to achieve SWAS (during resting FMRI)
5. SWAS: MRS, ASL, resting and task FMRI (pain + words)

7.2.5.4. Painful stimulation

We will either use mechanical, heat or electrical pain stimulation for the task-based FMRI assessments at loss of behavioural response and SWAS. We will apply pain stimulation at a maximum subjective pain intensity rating of 8 out of 10. This level will be assessed by a short thresholding procedure when the participants are fully awake and responsive in the scanner. This intensity level will be maintained for subsequent pain testing under anaesthesia at LOBR and SWAS.

We will use the Medoc Pathway contact heat thermode to induce heat pain, which is CE-approved is widely and routinely used for clinical diagnostic purposes. The limitations for contact heat/cold delivery are:

- minimum temperature: 0 degrees Celsius (deg C) (Medoc) for a stimulus duration of 3s, max. ramp time: 0.5 deg C/s)
- maximum temperature: 55 deg C for a stimulus duration of 3s (max. ramp time: 0.5 deg C/s)
- maximum size of stimulation site: 9cm^2.

For electrical pain, an electrode is applied to the skin surface, after it has been prepared with a commonly used cream that enhances conductance. Controlled current is applied only to this prepared surface area, without passing internally into the body. Equipment, such as Digitimer DS7A, Hertfordshire, UK, will be used to elicit a low-level of electrical output that is sufficient to induce a moderate-to-strong pain sensation. Equipment is certified for an output current of 0-100 mA, a source voltage of 100-400 V and stimulus durations between 50 μs to 2ms.

Mechanical pain is elicited through sensations related to touch. These can range from light touch to sharp pinprick are elicited using punctuate probes and von Frey hairs specifically designed to deliver a constant force to the skin surface. Furthermore, a purpose-built pressure device can be used to induce deep tissue pain (e.g., joint pain). None of these devices penetrate the skin. The maximum force delivered will be 512mN for punctate probes and von Frey hair and 250 N for the pressure device. There are no known side effects to any of these MRI–compatible stimulations.

The FMRIB pain laboratory has many years of experience using these painful stimulation devices (e.g. ethics references: C02.086, 05/Q1604/160, 06/Q1605/126, 09/H0604/90, 10/H0301/17 and CUREC Approved Procedure: IDREC_19_Version 4.0).

7.2.5.5. Auditory stimulation and modified isolated forearm test

Auditory stimulation will be delivered by Presentation (Neurobehavioral Systems) via the headphones that are used to communicate with the subject. We will use single-syllable words compiled from the MRC Psycholinguistics Database to determine the loss of behavioural response as in our previous study [21,22]. Subjects will respond using a two-option button box as to whether the two words presented are the ‘same’ or ‘different’. In order to assess for conscious content at loss of responsiveness and SWAS, we will use a modified form of the isolated forearm test [44], where we ask subjects to respond with a hand opening and closing to indicate whether they have perceived the stimulus and whether it was painful. In the same way as for the patient study, we will ask “[VOLUNTEER’S NAME], open and close your hand if you felt the stimulation” and “[VOLUNTEER’S NAME], open and close your hand if you felt pain”.

7.2.5.6. Recovery from anaesthesia

Emergence from anaesthesia will take place outside of the scanner bore. Volunteers will be removed from the bore and allowed to emerge naturally from the anaesthesia. They will remain on the scanner table in the presence of the anaesthetist while EEG data acquisition continues. The volunteers’ level of recovery will be assessed using the Modified Aldrete scoring system [49]. When participants are awake and oriented (as assessed by a Modified Aldrete score of at least 9), they will be administered the Nursing Delirium Screening Scale [48] and a semi-structured interview will be performed [50]. This will be recorded via the scanner intercom system (or alternative method) for scoring, evaluation and audio

transcription at a later time. Volunteers will then be transferred to an adjoining room where they will be supervised and have as much time to recover as they need. Individuals will be given a light meal afterwards and will discharged when they meet the criteria set out in Appendix D. Volunteers will be contacted later in the evening by the study anaesthetist to check that they are feeling well after the experiment.

In the event of an unexpected emergency incident, the emergency response team in the adjacent John Radcliffe Hospital covers FMRIB at the WIN. Emergency supplies (e.g. defibrillator) are available on site.

Due to individual variability in the susceptibility to anaesthesia, the time to complete this session will vary across volunteers. We expect the whole session to last between 4-5 hours in total, with delivery of sevoflurane anaesthesia for approximately 2 hours.

7.3. Discontinuation/Withdrawal of Participants from Study

Each participant has the right to withdraw from either study at any time. In addition, the Investigator may discontinue a participant from the study at any time if the Investigator considers it necessary for any reason including:

- Pregnancy
- Ineligibility (either arising during the study or retrospectively having been overlooked at screening)
- Significant protocol deviation
- Significant non-compliance with study requirements
- Withdrawal of Consent
- Loss to follow up

The reason for withdrawal will be recorded in the subject's CRF. Depending on the reason for withdrawal, data from the study will be included in analyses unless the subject requests for access to this data to be removed.

Any patients and healthy volunteers withdrawn on the basis of medical screening prior to experimental data collection will be replaced. If participants are withdrawn during data collection phases, they will not be replaced as we have increased the subject numbers accordingly to account this scenario.

7.4. Definition of End of Study

The end of the overall study will be the latter of either the follow-up of patients in Study 1 or the last visit for healthy volunteers in Study 2.

8. SAFETY REPORTING

The main safety concern for this study is the delivery of anaesthesia to our healthy volunteer participants. The side effects and complications that may occur when experiencing surgical anaesthesia are covered extensively by the Royal College of Anaesthesia (http://www.rcoa.ac.uk/system/files/PI-YAYA-COL-2014_1.pdf). Of these, the most common side-effects our healthy volunteers will potentially experience are feeling sick and/or vomiting, suffering from headache and experiencing some dizziness,

confusion or memory loss when emerging from the anaesthetic. These will be experienced by 1 in every 10 individuals. As these should be relatively short-lived expected reactions, they will be recorded on the individual's CRF if they occur.

More serious adverse events, such as heart attack, stroke, serious allergy to anaesthetic drugs and/or death are way more rare, and will be reported immediately as per the sections below. These complications affect individuals at a level of affecting 1 in 10,000 people. For example, in the worst case scenario, only five deaths occur for every million anaesthetics delivered in the UK.

Healthy volunteers will be followed up in the evening after completing the study to check for any adverse events. In the very unlikely event that the healthy volunteer participant should suffer any adverse effects related to the study after this time, they will be advised to contact the study research team using the details on the participant information sheet in the first instance.

Conversely, Individuals involved in the patient study who suffer from an adverse event following surgery will be expected to follow the normal clinical procedures in the first instance. We will ask to the NHS clinical team to keep us informed should this situation arise, and assess the impact (if any) of their study participation.

8.1. Definition of Serious Adverse Events

A serious adverse event is any untoward medical occurrence that:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- consists of a congenital anomaly or birth defect.

Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences.

The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

8.2. Reporting Procedures for Serious Adverse Events

A serious adverse event (SAE) occurring to a participant will be reported to the REC that gave a favourable opinion of the study if in the opinion of the Chief Investigator the event was 'related' (i.e. resulted from administration of any of the research procedures) and 'unexpected' in relation to those procedures. Reports of related and unexpected SAEs will be submitted within 15 working days of the Chief Investigator becoming aware of the event, using the HRA report of serious adverse event form (see HRA website).

9. STATISTICS AND ANALYSIS

9.1. Description of Statistical Methods

In order to ensure ongoing quality data acquisition, data will be analysed throughout the studies using the methods outlined below. Details of how the primary and secondary outcome measures will be assessed are also provided.

9.1.1. EEG data analyses

EEG analyses will be undertaken using Mathwork's Matlab software, Brain Products GMBH's BrainVision Analyzer 2 and the native software programs that are part of the sleep and clinical EEG recording systems. More customised statistical analyses maybe undertaken using Matlab and or IBM's SPSS statistics software. Concurrent EEG-MRI recordings result in artefacts in the EEG data that are caused by the switching of magnetic gradients during FMRI and ballistocardiographic artifacts related to cardiac activities. Having cleaned up these artifacts introduced by the scanner environment, simple spectral analyses of the EEG data will be performed in the first instance. Other global measures of whole-brain and sensor space brain connectivity will also be explored.

For example, EEG data collected during Study 2 will also be used to explore the relationship of the SWAS biomarker within the information theoretic approach to consciousness state. In collaboration with other LUMINOUS project partners (see section 13.1), the EEG data collected will be used to calculate measures of complexity such as the normalised version of the Lempel-Ziv complexity measures developed in [51] to assess the PCI. For this, EEG data will be source-reconstructed using a weighted minimum norm approach then thresholded and binarised with non-parametric bootstrap. For example, PCI measures will be compared to sensor-space slow wave measures (SWAS) in terms of their sensitivity to changes in drug concentrations.

With regard to the main study outcomes, we will apply our developed SWAS real-time Bayesian and post-hoc (off-line) models to fit sigmoid dose-response curves to each individual's data to identify SWAS. These will be used to indicate the success of our algorithm to achieve real-time titration and maintenance of an individual at SWAS. In particular, assessment of the inter- and intra-subject variability of the EEG at baseline, LOBR and SWAS, will give an indication of cortical (bi)stability at different anaesthetic depths.

9.1.2. MRI data analysis

Processing and analysis of functional images will be performed using standard methods available within the FMRIB software library MR image analysis package (www.fmrib.ox.ac.uk/fsl).

We will compare the resting and functional thalamocortical connectivity of sub-regions of thalamus at LOBR and SWAS with baseline. We will control for alterations in global and thalamic metabolism using the whole brain cerebral blood flow measures and specific magnetic resonance spectroscopy analyses of the thalamus at baseline and the SWAS end-point. Acquisition of a high-resolution anatomical (structural) scan will allow co-registration of the DTI and resting/task-related FMRI data collected during anesthesia. The structural and DTI data will enable us to perform multimodal within-subject analyses to address the wider study questions. For example, we will be able to assess the structural integrity of

specific thalamocortical pathways and relate this to alteration of resting and functional thalamocortical activity observed under anaesthesia.

We will directly compare the FMRI stimulus-evoked activity in response to the pain stimulus at baseline, LOBR and SWAS. We hope this will reproduce our earlier findings for a different anaesthetic agent (sevoflurane) that SWAS indicates perception loss to an incoming sensory stimulus [52]. In addition, the presence of 'conscious content' will be assessed by the presence/absence of response to the modified isolated forearm technique at LOBR and SWAS end-points. It is envisaged that there will be some residual conscious content at LOBR but this should be abolished at SWAS.

9.1.3. Psychophysical data analysis

Psychophysical data, including comparisons of sensory testing data across anaesthetic conditions and correlations between questionnaire data, will be analysed using standard statistical packages such as SPSS and Excel.

9.2. Number of Participants

For the healthy volunteer study, we estimate that we will need a sample size of 20 participants for analysis of our primary outcome based on our previous experience with neuroimaging of anaesthesia. This sample size is equivalent to or in excess of other contemporaneous FMRI experiments performed under anaesthesia [16,22,53]. However, it is anticipated that between 25-30 healthy volunteers will need to be recruited for the neuroimaging study to achieve completion by 20 volunteers. This will allow for subject attrition due to experimenter/anaesthetic safety concerns associated with anaesthesia delivery in the scanner (e.g. unacceptable cardiovascular instability or obstruction) or withdrawal by the subject for other reasons, such as claustrophobia in the scanner.

For study 1, we also estimate that we will require around 30 patients. We have calculated this as 10 participants per anaesthetic type - i.e. either sevoflurane or propofol anaesthesia with an extra 10 patients to allow for attrition. As mentioned previously, we expect the time taken to achieve SWAS to decrease with each patient with the subsequent optimisation and refinement of the model after each session. The sample size required to achieve this is very much an estimate as we are not sure how difficult it will be to apply the model in a real-time clinical setting at this stage.

10. DATA MANAGEMENT

Data will be handled according to the University of Oxford data protection policy, which is found at: http://www.admin.ox.ac.uk/councilsec/compliance/dataprotection/policy/. Dr Katie Warnaby, the Chief Investigator, and Professor Irene Tracey, head of the Nuffield Department of Clinical Neurosciences, will ensure that data specific to the project is processed in accordance with the UK's Data Protection Act and the National ethical review board guidelines.

Where possible, data management procedures will comply with the guidelines set out by European Union Horizon 2020 funding scheme: http://ec.europa.eu/research/participants/data/ref/h2020/grants_manual/hi/oa_pilot/h2020-hi-oa-data-mgt_en.pdf and those agreed by members of the LUMINOUS consortium.

10.1. Data Recording and Record keeping

Linked anonymisation will be used wherever and whenever possible. Individual patients and healthy volunteers will be given a unique identifier after consent and this will be used for all data records after inclusion. The project will generate several datasets per individual, each with different data types and formats. These include:

- Electroencephalographic (EEG) data in EDF or RAW format
- Magnetic resonance imaging data (MRI) in NIfTI or DICOM format
- Demographic data in comma separated value (CSV) file
- Behavioural data in CSV file
- Physiological data acquired in CSV or RAW format
- Encrypted audio files of interviews

A description document will be generated with the goal of making the data understandable to local researchers, those in the LUMINOUS consortium and other third parties (see sections 10.2.1 and 10.2.3). The description document will include the following sections:

- General information including the title of the dataset, institution of origin, ethics committee information and contact information for the PI at the University of Oxford
- Description of the experimental protocol
- Organizational information including study type, blinding and control group description
- Dataset information including coding of triggers, medication description, etc.

Additionally, each of the datasets will have an associated metadata file, which aims to establish a one-to-one association between the data files collected and its demographic, behavioural and technical details. The metadata is stored as a CSV format to ensure a common file type across all datasets within the LUMINOUS consortium.

10.2. Data access, sharing, preservation and archiving

Data may be accessed, shared, preserved and archived in three different ways. This will take place locally, within the LUMINOUS consortium and with third parties as part of open data access initiatives. Further details are outlined in the sections below:

10.2.1. Local users

Any subject identifying data will be stored either in locked filing cabinets only accessible to members of the research team, or locally on the secure WIN central file servers. Data acquired on laptop computers will also be copied to WIN servers. All data will be backed up on tapes provided by the WIN IT services. Anonymised research data will be stored indefinitely to enable further analyses should new techniques arise in the future. The WIN will retain personal identifiable data for at least 5 years.

10.2.2. LUMINOUS consortium

Data collection by the University of Oxford is independent to all other Luminous partners and their projects. Data will however be shared among LUMINOUS partners upon request in order to achieve the overarching project goals (see section 9.1.1 for an example). Data recorded during the execution of the

LUMINOUS project will remain confidential and only accessible to the members of the LUMINOUS consortium. The consortium leaders, Starlab will coordinate the data management, including its collection, storage and sharing between the LUMINOUS partners. Starlab have set up a data storage facility with a FTP protocol file server and a 100Mbps fiber line to facilitate data transfer. An access key will be provided by Starlab to allow LUMINOUS partners to access the data.

Confidentiality of research participants will be ensured in all ways. Strict anonymization protocols are in place at every institution where data is recorded. Personal identifiable data will not be shared with these parties, only anonymised data. Starlab has agreed to alert institutions to any aspect of the data that can potentially endanger participant's anonymisation.

For FTP access, LUMINOUS participants have read permissions only. Starlab members, as to conduct management tasks on the data storage facility, have read/write permissions. Upon submission to the data server, all data will go through a virus check and check for a rigorous anonymisation. The data collected during LUMINOUS will be stored by triplicate, so that three complete copies are stored physically separated from each other. These three storage spaces are

- First storage space will be accessible by password access by all LUMINOUS partners
- Second storage space will only be accessible by the repository manager for fast recovery
- Disaster prevention storage in a fireproof safe and only accessible only with key by IT manager

Data is replicated daily to a backup server and is saved to an external hard drive weekly. This weekly backup is stored in a fireproof safe box. Data collected during LUMINOUS will be available over 6 years after the completion of LUMINOUS project. To ensure integrity of the data on the long-term, periodic checks on the data accessibility will be done at both storage media (once a year), as well as periodic checks on whether old formats shall be transferred to new media or to new data formats.

10.2.3. Third party access

Data will be shared with other organisations both within and outside of the European Economic Area (EEA), most notably with our collaborators in New Zealand and the United States. Personal identifiable data will not be shared with these parties, only anonymised data. Consent for data sharing at this level will be obtained as part of the informed consent process.

Upon the signature of the Grant Agreement, all partners in the LUMINOUS consortium agreed to ascribe to the Open Research Data Pilot, allowing external sharing of the project data after final publication. Therefore, anonymised raw data and its associated metadata will ultimately be deposited on Zenodo (https://zenodo.org/). This is a tool from the Open Access Infrastructure for Research in Europe (OpenAIRE, https://www.openaire.eu), which is a public research data repository that links publications to their underlying research data. Using these methods, third parties will be able to access, mine, exploit, reproduce and disseminate our data and other data collected in LUMINOUS. OpenAIRE has requirements for data allocated on their services, and uploading it through Zenodo guarantees these standards. Data deposited in Zenodo for third party use will be secured according to Zenodo's security, backup and access control policies, which comply with EU directives. Data stored in Zenodo will also comply with their policies of preservation and archiving required from OpenAIRE. According to Zenodo information,

the servers are located at CERN Data Center, this CPD has multiple independent replicas which ensure the data integrity and accessibility.

In addition, we may also use the open access repository, Oxford Research Archive - Data (ORA-Data, http://www.bodleian.ox.ac.uk/bdlss/digital- services/data-archiving). This repository will allow the data to be accessed by any user for a period of 20 years.

11. QUALITY ASSURANCE PROCEDURES

The study may be monitored, or audited in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures. Direct access to the data will be granted to authorised representatives from the Sponsor and host institution for monitoring and/or audit of the study to ensure compliance with local, national or EU regulations.

12. ETHICAL AND REGULATORY CONSIDERATIONS

12.1. Declaration of Helsinki

The Investigator will ensure that this study is conducted in accordance with the principles of the Declaration of Helsinki.

12.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this study is conducted in accordance with relevant regulations and with Good Clinical Practice.

12.3. Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), and HRA for written approval. Prior to this, the above documents have received approval from the Ethical Advisory Board of the LUMINOUS Consortium.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

12.4. Reporting

The CI shall submit once a year throughout the study, or on request, an Annual Progress report to the REC Committee, HRA (where required) host organisation and Sponsor. In addition, an End of Study notification and final report will be submitted to the same parties.

12.5. Participant Confidentiality

The study will comply with the Data Protection Act, which requires data to be anonymised as soon as it is practical to do so.

Imaging data is automatically coded at source with an anonymisation code that cannot be directly linked to the volunteer. Any electronic data (e.g. EEG files, behavioural files, questionnaires) will be labelled with a code number rather than a name or initials. Data is accessed via a password and firewall protected server. With the written informed consent of the volunteer, fully anonymised data may be shared with other research institutions, including researchers outside of the EU, for other and future research studies.

Personal information (such as contact details) will be kept locally on a password-protected database on an encrypted machine or protected server. The keys linking codes to personal details will be kept in lockable filing cabinets with access only by the University researchers or locally in encrypted files on the secure WIN central file servers. Audio files for each participant will be separately encrypted with a personalised key that will be stored in the personal information database. These encrypted files will be retained in voice format until the end of the study. After that point, anonymised transcripts alone will be stored and the encryption key will be deleted from the personal information database when the audio transcription has been carried out. This personal identifiable data will be held for at least five years. Personal data may also be retained in a separate encrypted database if the participant agrees to be contacted for future studies.

Personal identifiable data may be viewed by regulatory bodies and designated individuals within the University of Oxford for the purposes of monitoring and auditing the research with the written consent of the volunteer. Personal data will not be shared with the LUMNOUS consortium or other third parties.

12.6. Expenses and Benefits

An overview of the general benefits of taking part in the studies outlined in the protocol was included in section 3.4. Details of expenses are included below:

12.6.1. Patient study

As the patient study should not involve any additional visits, we do not expect the patients to incur any additional costs. Should any visits in addition to normal care be required these will be reimbursed on production of receipts, or a provided mileage as appropriate.

12.6.2. Healthy volunteer study

Participants will receive £175 in total for their participation if they complete all sessions. They will receive £10 for screening during Visit 1, £25 for Visit 2, £50 for the overnight EEG sleep recording, and £90 for the anaesthesia session (Visit 3). Sessions will be paid on study completion or pro-rata in the case of withdrawal of the subject.

Reasonable travel expenses for any visits will also be reimbursed on production of receipts, or a mileage allowance provided as appropriate. Specifically, taxis will be paid for participant to travel to the John Radcliffe Hospital when they are wearing the sleep EEG kit (i.e. return home after Visit 2 and to attend Visit 3) and after they have received anaesthesia (i.e. return home after Visit 3). Volunteers will receive a free sandwich lunch before returning home after Visit 3.

12.7. Other Ethical Considerations

As discussed in broadly in section 3.4, we are not aware of any major ethical considerations that we have not managed appropriately in the study design and protocol. The ethical issues we have identified relate to the delivery of anaesthesia and the use of EEG and MRI neuroimaging techniques (see below):

12.7.1. Delivery of Anaesthesia

The risks and safety consideration of undergoing anaesthesia were described extensively in section 8. All healthy volunteers and patients will be fully able to give informed consent and screened carefully at medical screening. Through the studies' inclusion/exclusion criteria we have sought to recruit individuals at low risk of experiencing difficulties under anaesthesia, and having venous thromboembolism events due to prolonged immobilisation. Also, prior any MRI scanning, a formal airway assessment will also be carried out by a qualified anaesthetist to exclude individuals with a higher risk of obstruction under anaesthesia. Whilst, the expected levels of anaesthesia and their slow experimental delivery represent a minor risk to the volunteer's airway maintenance, we will additionally deliver air or oxygen to the participants to prevent hypoxia. Importantly, volunteers will be subjected to the same physiological monitoring as if they were experiencing day case surgery so their safety can be ensured at all times.

Any participants with private health insurance should be advised to contact their insurers to ensure that participation in this study does not contravene the terms and conditions of their policies.

12.7.2. Magnetic Resonance Imaging (MRI)

Magnetic Resonance Imaging (MRI) is safe and non-invasive and provides unsurpassed insight into the structure, organization and function of the living brain. The proposed MRI techniques, which use similar technology employed for clinical MRI examinations, are non-invasive and do not involve ionising radiation. While there are some risks associated with scanning some individuals (such as those with tattoos and some implants), these risks can be reduced when proper safety procedures are followed.

By measuring tiny magnetic signals from the blood or from nuclei (typically hydrogen) in different tissues, it is possible using MRI to characterize blood flow, tissue perfusion, metabolism and microstructure, as well as neuronal activity when the nervous system is at rest or performing a task. The specific types of MRI examinations that are performed vary in the nature of "pulse sequences", or the precise timing and duration of radio frequencies applied for the examination and the ways in which they are observed. Typically, different types of pulse sequences may be applied in a single imaging session to gain different types of information.

12.7.2.1. MRI risks to participants

All researchers at the WIN undergo Good Clinical Practice (GCP) training in order to be involved in research involving volunteers. All researchers involved with MRI are required to undergo annual MRI safety training – failure to undergo this training will automatically involve revocation of access to the centres. Scanning will be conducted by a HCPC registered radiographer or another senior and appropriately trained scan operator.

i) Comfort:

Certain 3T and 7T MRI sequences can be very noisy so participants will be given earplugs and or ear-defenders.

The enclosed space of the scanner can induce feelings of claustrophobia. All operators and radiographers are accustomed to dealing with participants who may be claustrophobic and have a variety of strategies to employ with people who exhibit feelings of claustrophobia but who still wish to participate in the study. Participants will be introduced carefully to the scanner and allowed to leave at any stage. Whilst in the scanner participants have easy access to a call button should they wish to stop the scan or speak with the radiographer or operator.

In order to reduce as far as possible the risks associated with MRI scanning, participants will be carefully screened for surgical or other implanted metallic devices as a result of surgery or accidents every time they attend for a scan. Furthermore, they will change into pocket less surgical scrubs for their scan.

Lying on the scanner table for prolonged times can induce temporary lower back pain. MRI-compatible pads and cushions may be used to improve participant comfort.

ii) Ferromagnetic Objects:

Ferromagnetic objects will not be permitted in the scanner room. Any stimulation or monitoring equipment with metallic components must be MRI safe or MRI compatible.

Any researchers entering the magnet environment will have undertaken annual MRI safety training and completed an annual Visitor MRI Safety Screening form to ensure maximal awareness and safety around the scanner. The research volunteers will complete a Volunteer MRI Safety Screening form for each visit.

12.7.2.2. Incidental findings

Occasionally, MRI studies identify abnormal anatomy or pathology in healthy volunteers. An incidental finding may be detected either at the time the scan is collected or may be identified some time later, potentially months or even years later.

In the unlikely event of seeing any structural abnormalities on an MRI scan, a clinical specialist will check the scan. If the specialist feels that the abnormality was medically important, they will discuss the implications with the participant and arrange for further investigations as necessary. Participants will not be informed unless the doctor considers the finding has clear implications for their current or future health. It is important to note that scans are not carried out for diagnostic purposes, and therefore the scans are not a substitute for a clinical appointment. Rather, the scans are intended for research purposes only.

If an abnormality is detected, the WIN (FMRIB/OCMR/OHBA) SOP – Dealing with Neuro-Incidental Findings will be followed. The Principal Investigator would alert the Contact Radiographer who will make an initial assessment as to whether the abnormality may reflect a scanner artefact, or is of a trivial nature. Once an incidental finding is suspected, the Contact Radiographer will inform the Contact Neurologist as soon as possible, who will meet with the Contact Radiologist at the John Radcliffe Hospital and together decide whether the finding warrants further clinical investigation. In this eventuality, the Contact Neurologist would contact the volunteer directly and the appropriate action be discussed. The

Contact Neurologist maintains a database with anonymised summary information on the outcomes of all referrals.

12.7.2.3. MRI risks to researchers

As described above, any metallic objects in or around the magnet will be designated MRI Conditional or MRI Safe to preclude projectile risks.

12.7.3. EEG

EEG will be used in both the patient and healthy volunteer studies. This is a very safe technique that has been used for nearly 100 years. EEG recording has been used safely for many years, and we are aware of no cases of adverse events. EEG equipment comes from certified suppliers of medical equipment, who are obliged by law to adhere to published guidelines on electrical and mechanical safety (IEC-601).

During the set-up of the EEG, participants are asked to indicate if they feel any discomfort, in which case the procedure is stopped. It is possible to pause the procedure if a participant needs to take a break or visit the bathroom, or if a fire alarm goes off.

Brain potentials vary widely from individual to individual. Researchers undertake not to make any judgemental comments on the type of brain potentials seen in individual participants, to avoid causing unnecessary anxiety, e.g. the researcher should not make a comment such as “you’ve only got very small brain responses”.

Hygienic use of the equipment will be ensured by soaking the EEG sensors, caps and the instruments used in applying EEG gel in disinfectant solution after each use. In the majority of cases, participants wash their hair to remove gel at the end of the session, and freshly laundered towels are provided in each case.

12.7.3.1. EEG Risks to researchers

Again, the main way to avoid risk is to adhere to a regime of hygiene. Hands are washed after any contact with the scalp of a participant.

12.7.4. Simultaneous EEG and MRI

Simultaneous EEG-fMRI offers a particularly powerful tool when it is important to determine both the time-scale and the spatial location in the brain where a signal is processed. The only difference from stand-alone EEG measurements is that simultaneous acquisitions use specialized MRI-compatible EEG caps to take measurements during the MRI scan. MRI, EEG and combined fMRI-EEG imaging methods do not cause pain or harm to the participant. EEG acquisitions can take place either in the scanner or outside of the scanner.

EEG equipment to be used in conjunction with MRI scans will be in all cases certified as ‘MR conditional’ up to 3T field strengths and used with WIN Centre approved scan sequences. As before, hygienic use of the equipment will be ensured by soaking the EEG sensors, caps and the instruments used in applying EEG gel in disinfectant solution after each use. In the majority of cases, participants will wash their hair

to remove gel at the end of each session (facilities are in place within the centres for this purpose) and MRI headrests are covered with clean paper towels for every participant.

12.7.5. Painful stimulation

The application of sensory stimuli such as thermal and mechanical stimulation during scanning will cause some transient pain or discomfort, and can cause temporary redness of the skin. This will be short-lived, and participants will be able to stop the experiment at any time if they wish. Participants will be made aware of this during the consent process. Participants will be thresholded on the basis of their individual pain perception when they are awake and responsive. As stated in the study hypotheses and goals, the delivery of sevoflurane anaesthesia is expected to reduce pain sensitivity.

13. FINANCE AND INSURANCE

13.1. Funding

The two studies outlined in this protocol are funded by a Horizon 2020 European Union (EU) Future Emerging Technologies Open Research Grant (reference H2020-FETOPEN-2014-2015-RIA Grant agreement No 686764). This research grant funds a broader programme of consciousness research from a consortium of eight EU institutions. The University of Oxford provide the anaesthesia expertise on the project. The consortium operates under the acronym of LUMINOUS, with the overarching research grant is entitled “Studying, Measuring and Altering Consciousness through information theory in the electrical brain”.

13.2. Insurance

The University has a specialist insurance policy in place, which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd’s of London).

14. PUBLICATION POLICY

Study results may be written up for publication in peer-reviewed scientific journals, presented at scientific conferences (in abstract or presentation formats), entered into fully-anonymised repositories of imaging data, submitted as part of course degrees and may form part of grant applications. In all cases, results will be fully anonymised and not contain any data that could be linked to the volunteers.

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge that the study was funded by a Horizon 2020 European Union (EU) Future Emerging Technologies Research Grant. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

15. REFERENCES

1. Leslie, K., Chan, M.T.V., Myles, P.S., Forbes, A., and McCulloch, T.J. (2010). Posttraumatic Stress Disorder in Aware Patients from the B-Aware Trial: Anesth. Analg. *110*, 823–828.
2. Sessler, D.I., Sigl, J.C., Kelley, S.D., Chamoun, N.G., Manberg, P.J., Saager, L., Kurz, A., and Greenwald, S. (2012). Hospital stay and mortality are increased in patients having a “triple low” of low blood pressure, low bispectral index, and low minimum alveolar concentration of volatile anesthesia. Anesthesiology *116*, 1195–1203.
3. Leslie, K., Myles, P.S., Forbes, A., and Chan, M.T.V. (2010). The effect of bispectral index monitoring on long-term survival in the B-aware trial. Anesth. Analg. *110*, 816–822.
4. Monk, T.G., Saini, V., Weldon, B.C., and Sigl, J.C. (2005). Anesthetic Management and One-Year Mortality After Noncardiac Surgery. Anesth. Analg. *100*, 4–10.
5. Schneider, G., Hollweck, R., Ningler, M., Stockmanns, G., and Kochs, E.F. (2005). Detection of consciousness by electroencephalogram and auditory evoked potentials. Anesthesiology *103*, 934–943.
6. Johansen, J.W. (2006). Update on bispectral index monitoring. Best Pract. Res. Clin. Anaesthesiol. *20*, 81–99.
7. Messina, A.G., Wang, M., Ward, M.J., Wilker, C.C., Smith, B.B., Vezina, D.P., and Pace, N.L. (2016). Anaesthetic interventions for prevention of awareness during surgery. In Cochrane Database of Systematic Reviews, The Cochrane Collaboration, ed. (Chichester, UK: John Wiley & Sons, Ltd). Available at: http://doi.wiley.com/10.1002/14651858.CD007272.pub2 [Accessed October 26, 2016].
8. Shepherd, J., Jones, J., Frampton, G., Bryant, J., Baxter, L., and Cooper, K. (2013). Clinical effectiveness and cost-effectiveness of depth of anaesthesia monitoring (E-Entropy, Bispectral Index and Narcotrend): a systematic review and economic evaluation. Health Technol. Assess. Winch. Engl. *17*, 1–264.
9. White, N.S., and Alkire, M.T. (2003). Impaired thalamocortical connectivity in humans during general-anesthetic-induced unconsciousness. NeuroImage *19*, 402–11.
10. Kaisti, K.K., Metsahonkala, L., Teras, M., Oikonen, V., Aalto, S., Jaaskelainen, S., Hinkka, S., and Scheinin, H. (2002). Effects of surgical levels of propofol and sevoflurane anesthesia on cerebral blood flow in healthy subjects studied with positron emission tomography. Anesthesiology *96*, 1358–70.
11. Dang-Vu, T.T. (2012). Neuronal Oscillations in Sleep: Insights from Functional Neuroimaging. NeuroMolecular Med. Available at: http://www.springerlink.com/index/10.1007/s12017-012-8166-1 [Accessed July 18, 2012].
12. Mashour, G.A., and Alkire, M.T. (2013). Consciousness, Anesthesia, and the Thalamocortical System: Anesthesiology *118*, 13–15.
13. Rivolta, D., Heidegger, T., Scheller, B., Sauer, A., Schaum, M., Birkner, K., Singer, W., Wibral, M., and Uhlhaas, P.J. (2015). Ketamine Dysregulates the Amplitude and Connectivity of High-Frequency Oscillations in Cortical-Subcortical Networks in Humans: Evidence From Resting-State Magnetoencephalography-Recordings. Schizophr. Bull. *41*, 1105–1114.
14. Akeju, O., Loggia, M.L., Catana, C., Pavone, K.J., Vazquez, R., Rhee, J., Ramirez, V.C., Chonde, D.B., Izquierdo-Garcia, D., Arabasz, G., *et al.* (2015). Disruption of thalamic functional connectivity is a neural correlate of dexmedetomidine-induced unconsciousness. eLife *3*, e04499.
15. Liu, X., Lauer, K.K., Ward, B.D., Li, S.-J., and Hudetz, A.G. (2013). Differential effects of deep sedation with propofol on the specific and nonspecific thalamocortical systems: a functional magnetic resonance imaging study. Anesthesiology *118*, 59–69.
16. Jordan, D., Ilg, R., Riedl, V., Schorer, A., Grimberg, S., Neufang, S., Omerovic, A., Berger, S., Untergehrer, G., Preibisch, C., *et al.* (2013). Simultaneous electroencephalographic and functional magnetic resonance imaging indicate impaired cortical top-down processing in association with anesthetic-induced unconsciousness. Anesthesiology *119*, 1031–1042.
17. Bonhomme, V., Boveroux, P., Brichant, J.F., Laureys, S., and Boly, M. (2012). Neural correlates of consciousness during general anesthesia using functional magnetic resonance imaging (fMRI). Arch Ital Biol *150*, 155–163.

18. Mhuircheartaigh, R.N., Rosenorn-Lanng, D., Wise, R., Jbabdi, S., Rogers, R., and Tracey, I. (2010). Cortical and Subcortical Connectivity Changes during Decreasing Levels of Consciousness in Humans: A Functional Magnetic Resonance Imaging Study using Propofol. J. Neurosci. *30*, 9095–9102.
19. Lee, U., Mashour, G.A., Kim, S., Noh, G.-J., and Choi, B.-M. (2009). Propofol induction reduces the capacity for neural information integration: Implications for the mechanism of consciousness and general anesthesia. Conscious. Cogn. *18*, 56–64.
20. Lee, U., Kim, S., Noh, G.-J., Choi, B.-M., Hwang, E., and Mashour, G.A. (2009). The directionality and functional organization of frontoparietal connectivity during consciousness and anesthesia in humans. Conscious. Cogn. *18*, 1069–1078.
21. Warnaby, C.E., Seretny, M., Ní Mhuircheartaigh, R., Rogers, R., Jbabdi, S., Sleigh, J., and Tracey, I. (2016). Anesthesia-induced Suppression of Human Dorsal Anterior Insula Responsivity at Loss of Volitional Behavioral Response. Anesthesiology *124*, 766–778.
22. Ni Mhuircheartaigh, R., Warnaby, C., Rogers, R., Jbabdi, S., and Tracey, I. (2013). Slow-Wave Activity Saturation and Thalamocortical Isolation During Propofol Anesthesia in Humans. Sci. Transl. Med. *5*, 208ra148-208ra148.
23. Murphy, M., Bruno, M.A., Riedner, B.A., Boveroux, P., Noirhomme, Q., Landsness, E.C., Brichant, J.F., Phillips, C., Massimini, M., and Laureys, S. (2011). Propofol anesthesia and sleep: a high-density EEG study. Sleep *34*, 283.
24. Koch, C., Massimini, M., Boly, M., and Tononi, G. (2016). Neural correlates of consciousness: progress and problems. Nat. Rev. Neurosci. *17*, 307–321.
25. Steriade, M. (2006). Grouping of brain rhythms in corticothalamic systems. Neuroscience *137*, 1087–1106.
26. Leslie, K.A.-M., P.S. AU-Forbes, A. AU-Chan, M.T. (2010). The effect of bispectral index monitoring on long-term survival in the B-aware trial. Anesth Analg *110*, 816–22.
27. Leslie, K.A.-C., M.T. AU-Myles, P.S. AU-Forbes, A. AU-McCulloch, T.J. (2010). Posttraumatic stress disorder in aware patients from the B-aware trial. Anesth Analg *110*, 823–8.
28. Sessler, D.I.A.-S., J.C. AU-Kelley, S.D. AU-Chamoun, N.G. AU-Manberg, P.J. AU-Saager, L. AU-Kurz, A. AU-Greenwald, S. (2012). Hospital stay and mortality are increased in patients having a “triple low” of low blood pressure, low bispectral index, and low minimum alveolar concentration of volatile anesthesia. Anesthesiology *116*, 1195–203.
29. Warnaby CE, Sleigh JW, Hight D, Jbabdi S, and Tracey I Neural Inertia and Slow Wave Activity Saturation Identified during Surgical Anesthesia in Humans. Anesthesiology *accepted*.
30. McKay, I.D.H., Voss, L.J., Sleigh, J.W., Barnard, J.P., and Johannsen, E.K. (2006). Pharmacokinetic-Pharmacodynamic Modeling the Hypnotic Effect of Sevoflurane Using the Spectral Entropy of the Electroencephalogram: Anesth. Analg. *102*, 91–97.
31. Law, C.J., Jacobson, G.M., Kluger, M., Chaddock, M., Scott, M., and Sleigh, J.W. (2014). Randomized controlled trial of the effect of depth of anaesthesia on postoperative pain. Br. J. Anaesth. *112*, 675–680.
32. Hight, D.F., Dadok, V.M., Szeri, A.J., GarcÃ-a, P.S., Voss, L., and Sleigh, J.W. (2014). Emergence from general anesthesia and the sleep-manifold. Front. Syst. Neurosci. *8*. Available at: http://journal.frontiersin.org/Journal/10.3389/fnsys.2014.00146/full [Accessed January 13, 2015].
33. Dang-Vu, T.T., Bonjean, M., Schabus, M., Boly, M., Darsaud, A., Desseilles, M., Degueldre, C., Balteau, E., Phillips, C., Luxen, A., *et al.* (2011). Interplay between spontaneous and induced brain activity during human non-rapid eye movement sleep. Proc. Natl. Acad. Sci. *108*, 15438–15443.
34. Franks, N.P. (2008). General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. Nat. Rev. Neurosci. *9*, 370–386.
35. Hill, J., Treasure, T., and on behalf of the Guideline Development Group (2010). Reducing the risk of venous thromboembolism (deep vein thrombosis and pulmonary embolism) in patients admitted to hospital: summary of the NICE guideline. Heart *96*, 879–882.
36. Mapleson, W.W. (1996). Effect of age on MAC in humans: a meta-analysis. Br. J. Anaesth. *76*, 179–185.

37. Liu, R.S.N., Lemieux, L., Bell, G.S., Sisodiya, S.M., Shorvon, S.D., Sander, J.W. a. S., and Duncan, J.S. (2003). A longitudinal study of brain morphometrics using quantitative magnetic resonance imaging and difference image analysis. NeuroImage *20*, 22–33.
38. Kil, H.K., Kim, W.O., Chung, W.Y., Kim, G.H., Seo, H., and Hong, J.-Y. (2012). Preoperative anxiety and pain sensitivity are independent predictors of propofol and sevoflurane requirements in general anaesthesia. Br. J. Anaesth. *108*, 119–125.
39. Tellegen, A., and Atkinson, G. (1974). Openness to absorbing and self-altering experiences (" absorption"), a trait related to hypnotic susceptibility. J. Abnorm. Psychol. *83*, 268.
40. Glisky, M.L., Tataryn, D.J., Tobias, B.A., Kihlstrom, J.F., and McConkey, K.M. (1991). Absorption, openness to experience, and hypnotizability. J. Pers. Soc. Psychol. *60*, 263.
41. Spielberger, C. (1983). Manual for the State-Trait Anxiety Inventory.
42. Moerman, N., van Dam, F.S., Muller, M.J., and Oosting, H. (1996). The Amsterdam Preoperative Anxiety and Information Scale (APAIS). Anesth. Analg. *82*, 445–451.
43. Buysse, D.J., Reynolds, C.F., Monk, T.H., Berman, S.R., and Kupfer, D.J. (1989). The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. *28*, 193–213.
44. Sanders, R.D., Gaskell, A., Raz, A., Winders, J., Stevanovic, A., Rossaint, R., Boncyk, C., Defresne, A., Tran, G., Tasbihgou, S., *et al.* (2017). Incidence of Connected Consciousness after Tracheal Intubation: A Prospective, International, Multicenter Cohort Study of the Isolated Forearm Technique. Anesthesiology *126*, 214–222.
45. Gaudreau, J.-D., Gagnon, P., Harel, F., Tremblay, A., and Roy, M.-A. (2005). Fast, Systematic, and Continuous Delirium Assessment in Hospitalized Patients: The Nursing Delirium Screening Scale. J. Pain Symptom Manage. *29*, 368–375.
46. Brice, D.D., Hetherington, R.R., and Utting, J.E. (1970). A simple study of awareness and dreaming during anaesthesia. Br. J. Anaesth. *42*, 535–542.
47. Asai, T., Kanayama, N., Imaizumi, S., Koyama, S., and Kaganoi, S. (2016). Development of Embodied Sense of Self Scale (ESSS): Exploring Everyday Experiences Induced by Anomalous Self-Representation. Front. Psychol. *7*. Available at: http://journal.frontiersin.org/Article/10.3389/fpsyg.2016.01005/abstract [Accessed April 10, 2017].
48. Levenson, H. (1973). Multidimensional locus of control in psychiatric patients. J. Consult. Clin. Psychol. *41*, 397–404.
49. Fritz, B.A., Rao, P., Mashour, G.A., Abdallah, A.B., Burnside, B.A., Jacobsohn, E., Zhang, L., and Avidan, M.S. (2013). Postoperative Recovery with Bispectral Index versus Anesthetic Concentration-Guided Protocols. Anesthesiology.
50. Noreika, V., Jylhänkangas, L., Móró, L., Valli, K., Kaskinoro, K., Aantaa, R., Scheinin, H., and Revonsuo, A. (2011). Consciousness lost and found: Subjective experiences in an unresponsive state. Brain Cogn. *77*, 327–334.
51. Casali, A.G., Gosseries, O., Rosanova, M., Boly, M., Sarasso, S., Casali, K.R., Casarotto, S., Bruno, M.-A., Laureys, S., Tononi, G., *et al.* (2013). A Theoretically Based Index of Consciousness Independent of Sensory Processing and Behavior. Sci. Transl. Med. *5*, 198ra105–98ra105.
52. Warnaby, CE, Sleigh, JW, Hight, D, Garcia, PS, Jbabdi, S, and Tracey, I Modelling Slow Wave Activity Saturation in Individuals during Surgical Anesthesia and its Relationship with their Emergence Trajectory. Submiss.
53. Palanca, B.J.A., Mitra, A., Larson-Prior, L., Snyder, A.Z., Avidan, M.S., and Raichle, M.E. (2015). Resting-state Functional Magnetic Resonance Imaging Correlates of Sevoflurane-induced Unconsciousness. Anesthesiology *123*, 346–356.

Appendix A SCHEDULE OF PROCEDURES - PATIENT STUDY (STUDY 1)

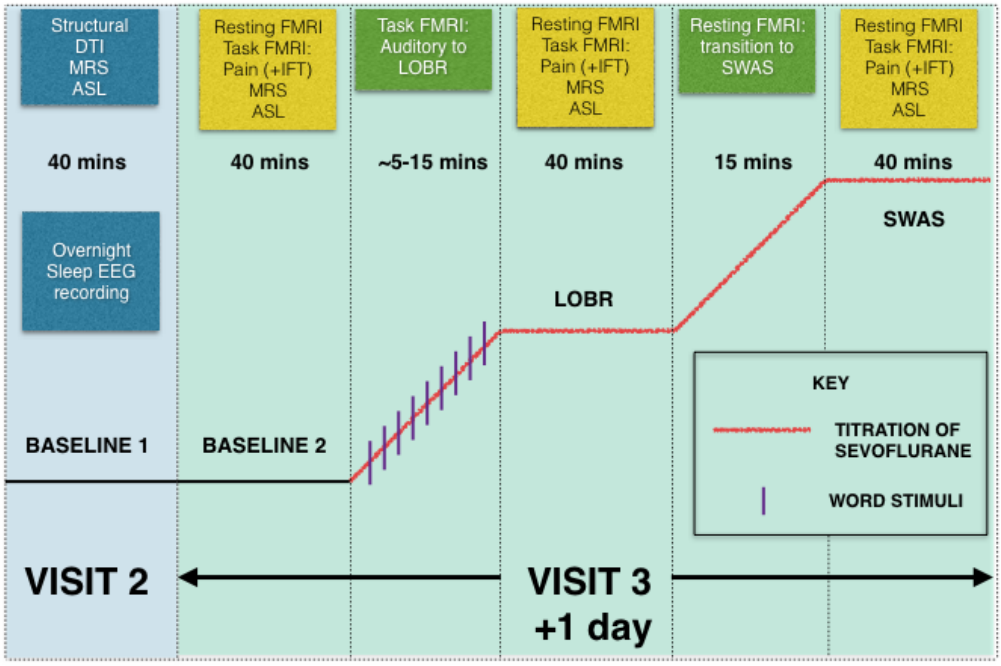
	Pre-op			Day of surgery			Post-op
	Outpatients	**Pre-operative assessment**	**Telephone interview**	**Pre-op**	**Peri-op**	**Post-op**	**3-4 weeks**
Screening by clinical team	✓	✓					
Study explanation and eligibility assessment by study team	✓	✓	✓				
Informed consent		✓		✓			
Allocation to propofol or sevoflurane anaesthesia				✓			
EEG monitoring applied				✓			
Questionnaires: anxiety, sleep, absorbance, etc.				✓			
Anaesthetic delivery to SWAS				✓			
EEG data acquisition				✓	✓	✓	
Physiological data recording				✓	✓	✓	
Questionnaire/interview: Intraoperative experiences						✓	(✓)
Questionnaire: patient satisfaction							✓

Appendix B SCHEDULE OF PROCEDURES - HEALTHY VOLUNTEER STUDY (STUDY 2)

Procedures		**Visits**			
		Visit 1	**Visit 2 (MRI on Day 1)**		**Visit 3 (MRI on Day 2)**
	Eligibility screening	**Medical Screening**	**Baseline**	**Overnight**	**Anaesthesia**
Participant information received and understood	✓	✓			
Eligibility assessment	✓	✓			
MR Screening form completed	✓	✓			
Informed consent		✓			
Explanation of study procedures and equipment		✓			
Consultation with anaesthetist		✓			
Demographics		✓			
Medical History		✓			
Magnetic resonance imaging			✓		✓
Questionnaires			✓		✓
Delivery of anaesthesia					✓
EEG monitoring				✓	✓
Physiological monitoring			✓	✓	✓
Adverse event assessments: participant follow-up					✓

Appendix C OVERVIEW OF EXPERIMENTAL SESSIONS - STUDY 2

The experimental study procedures that take place during the MRI Visits 2 and 3 are outline below. These visits take place after the medical screening visit (Visit 1).



Appendix D DISCHARGE CRITERIA – STUDY 2

	Circle one
Alert and oriented	Yes/No
Vital signs within normal limits	Yes/No
Taking and tolerating fluids and foods	Yes/No
Able to stand and walk unaided	Yes/No
Monitoring equipment removed	Yes/No
IV removed	Yes/No
Confirmed accompaniment at home for evening	Yes/No
Contact details confirmed for follow up	Yes/No
Investigator contact details with subject	Yes/No

Appendix E AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made