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Foundations of human consciousness: Imaging the twilight zone

Abbreviated Title: Imaging connected consciousness

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M.S.: Consultancy and contract research relationships with Orion Pharma, the original manufacturer of dexmedetomidine. Orion Pharma was not involved in the planning or execution of the current study. M.S. was also listed as inventor in Orion Pharma's US Patent Application US5344840A from 1993, "4-substituted imidazole derivatives useful in perioperative care", now expired. M.S. declares no current competing financial interests in the study. Other authors declare no competing financial interests.

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Abstract

What happens in the brain when conscious awareness of the surrounding world fades? We manipulated consciousness in two experiments in a group of healthy males and measured brain activity with positron emission tomography. Measurements were made during wakefulness, escalating and constant levels of two anesthetic agents (Experiment 1, n=39) and during sleep-deprived wakefulness and Non-Rapid Eye Movement sleep (Experiment 2, n=37). In Experiment 1, the subjects were randomized to receive either propofol or dexmedetomidine until unresponsiveness. In both experiments, forced awakenings were applied to achieve rapid recovery from an unresponsive to a responsive state, followed by immediate and detailed interviews of subjective experiences during the preceding unresponsive condition. Unresponsiveness rarely denoted unconsciousness, as the majority of the subjects had internally generated experiences. Unresponsive anesthetic states and verified sleep stages, where a subsequent report of mental content included no signs of awareness of the surrounding world, indicated a disconnected state. Functional brain imaging comparing responsive and connected *vs.* unresponsive and disconnected states of consciousness during constant anesthetic exposure revealed that activity of the thalamus, cingulate cortices and angular gyri are fundamental for human consciousness. These brain structures were affected independent from the pharmacologic agent, drug concentration and direction of change in the state of consciousness. Analogous findings were obtained when consciousness was regulated by physiological sleep. State-specific findings were distinct and separable from the overall effects of the interventions, which included widespread depression of brain activity across cortical areas. These findings identify a central core brain network critical for human consciousness.

Significance Statement

Trying to understand the biological basis of human consciousness is currently one of the greatest challenges of neuroscience. While the loss and return of consciousness regulated by anesthetic drugs and physiological sleep are employed as model systems in experimental studies on consciousness, previous research results have been confounded by drug effects, by confusing behavioral "unresponsiveness" and internally generated consciousness, and by comparing brain activity levels across states that differ in several other respects than only consciousness. Here, we present carefully designed studies that overcome many previous confounders and for the first time reveal the neural mechanisms underlying human consciousness and its disconnection from behavioral responsiveness, both during anesthesia and during normal sleep, and in the same study subjects.

Introduction

Experimental anesthesia and natural sleep are powerful research tools in the study of human consciousness (Fiset et al., 1999; Alkire et al., 2000; Horovitz et al., 2009; Boveroux et al., 2010; Långsjö et al., 2012; Liu et al., 2013; Akeju et al., 2014; Warnaby et al., 2016). Neural correlates of consciousness are often claimed to be found by comparing brain activity data collected during two states: wakefulness and a presumed unconscious state. This paradigm is, however, controversial in two fundamental ways. First, the state of consciousness is often defined by behavior, i.e., unconsciousness by lack of meaningful responses to external stimuli. Unresponsiveness does not, however, ensure unawareness (Owen et al., 2006, Huang et al., 2018) or absence of internally generated experiences (Brice et al., 1970; Radek et al., 2018) and is, thus, by definition, not unconsciousness. Indeed, a conscious state can be defined as having experiences, also referred to as contents of consciousness. Yet, experimental studies rarely characterize the explored states explicitly or beyond behavioral properties (Bonhomme et al., 2019). In a *connected* state, such as during normal wakefulness, the contents of consciousness are modulated by incoming sensory information, resulting in conscious awareness of actual physical stimuli. In a *disconnected* state, the contents of consciousness are seldom related to incoming sensory information and typically consist of only internally generated experiences. Unconsciousness, i.e., absence of experiences, also represents a disconnected state. Table 1 summarizes the characteristics of these conditions (modified from Sanders et al., 2012; Bonhomme et al., 2019), clarifying the multi-dimensional nature of human consciousness. Importantly, a disconnected state should be viewed as characteristic for successful general anesthesia, and complete unconsciousness is difficult to confirm in experimental settings.

The second problem concerning experimental anesthesia as a proxy to explore consciousness is the assumption that differences between wakefulness and (presumed) unconsciousness would straightforwardly reflect the neural correlates of consciousness (Scheinin et al., 2018b). This is not

the case, as anesthetic drugs have sedative and other direct and indirect effects on the brain, which may affect the interpretations of the obtained data. Pharmacologic limitations can be resolved, e.g., by exploring physiological sleep, but drowsiness and sleep pressure also affect brain activity independently of major changes in the state of consciousness. In the current study, we aimed to separate changes in brain activity related specifically to consciousness from the overall effects of anesthesia and sleep. We applied novel experimental approaches to tackle previous limitations and to address three main questions: (i) what are the neural correlates of connected consciousness, as assessed by identifying the specific differences in brain activity between connected and disconnected states of consciousness, (ii) are anesthesia and physiologic sleep similar or different in this respect, and (iii) are the brain areas affected by transitions from connected to disconnected and from disconnected to connected states the same or different? We used positron emission tomography (PET) imaging to measure brain activity, reflected by changes in regional cerebral blood flow (rCBF) in two separate experiments in the same group of healthy subjects. Measurements were made during wakefulness, step-wise escalating and constant levels of two anesthetic agents (Experiment 1) and during sleep-deprived wakefulness and Non-Rapid Eye Movement (NREM) sleep stages (Experiment 2).

In both experiments, two sets of analyses were carried out: The first aimed to discover overall effects of anesthesia and sleep by comparing different doses of the drugs and different sleep stages to awake baseline. The second aimed to identify state-specific patterns in brain activity. Here, only within-subject *connected* and *disconnected* states of consciousness, with minimal confounding effects, were compared. Maintained responsiveness to external auditory stimuli and an awake sleep-deprived state indicated a connected state. Unresponsive anesthetic states and verified sleep stages, where a subsequent immediate report of mental content included no signs of awareness of the surrounding world (see Material and Methods) indicated a disconnected state.

Material and Methods

Subjects. The study was approved by the Ethics Committee of the Hospital District of Southwest Finland and the Finnish Medicines Agency Fimea, and registered at ClinicalTrials.gov (identifier NCT01889004). Altogether, forty 20−30-yr old healthy, ASA 1 (according to the American Society of Anesthesiologists physical status classification system), right-handed male volunteers were recruited. Only male subjects were included because of the radiation exposure related to positron emission tomography (PET) imaging. All subjects were interviewed and thoroughly examined by a licensed physician (A.S.). A standard 12-lead electrocardiogram (ECG) and blood and urine samples were analyzed to confirm the subjects´ health status. Exclusion criteria included any somatic illness, regular medication or drug allergy, history of any psychiatric disorder or substance abuse, cardiac arrhythmias, hearing impairment, propensity to severe nausea in connection to anesthesia, blood donation in the preceding 90 days, prior participation in a PET/SPECT study, any contraindication to magnetic resonance imaging (MRI), detected unsuitability based on initial electrophysiological measurements, detected unsuitability based on anatomical MRI scans and pathological findings in laboratory tests or positive urine drug screen result. All subjects provided written informed consent according to the Declaration of Helsinki.

Experimental designs and study objectives. Our aim was to investigate human consciousness in two separate experiments, utilizing PET imaging, two different anesthetic agents and physiological sleep. Functional brain imaging data were obtained during escalating and constant levels of anesthesia, in different states of consciousness (responsive and connected *vs.* unresponsive and disconnected) and in different sleep stages. Scans were compared within and between subjects to identify brain regions fundamental for regulation of human consciousness. Our experimental designs tried to bypass some previous limitations related to drug administration and heterogeneous dosing schemes. Specifically, we eliminated the confounding sedative and possible other drug-induced effects as well as the confounding effect of sleep pressure on brain activity. We also

extended the assessment of the state of consciousness beyond behavior and conducted interviews to verify the phenomenal state of the subjects, i.e., the presence or absence of experiences during unresponsiveness.

Subjects were investigated during drug-induced anesthesia (exposure to either propofol or dexmedetomidine) and during physiological sleep. In both experiments, brain activity was measured using functional PET imaging of rCBF, using ^{15}O-labelled H_2O as tracer. In Experiment 1 (n=39), scans were obtained during escalating and constant anesthetic levels, which represented different states of consciousness driven by forced awakenings from an unresponsive state. In Experiment 2 (n=37), the same subjects were studied on the average 18 weeks later, and brain activity, reflected by changes in rCBF, was measured during sleep deprivation and NREM sleep stages N1, N2 and N3. Experiment 1 (anesthesia) was open and randomized. Permuted blocks were applied to achieve balanced groups across treatments. Detailed study outlines of both experiments are described in "Anesthesia Study" and "Sleep Study" and schematically illustrated in Figure 1.

Anesthesia study (Experiment 1). The subjects abstained from the use of alcohol and any medication for at least 48 h and fasted overnight prior to the experiment. Two forearm veins were cannulated for administration of study drugs and the PET radiotracer and for blood sampling. Intravenous anesthetics, propofol (Propofol Lipuro 10 mg/ml, B. Braun) or dexmedetomidine (Dexdor 100 µg/ml, Orion Pharma) were administered using target-controlled infusions (TCI) with previously described pharmacokinetic parameters (Marsh et al., 1991; Talke et al., 2003). A Harvard 22 syringe pump (Harvard Apparatus, South Natick, MA) and a portable computer running Stanpump software was used for drug administration (by Steven L. Schafer, MD, www.opentci.org/code/stanpump). Plasma targets were used. Electroencephalogram (EEG) was recorded with a 64 channel Ag/AgCl active electrode cap (EasyCap GmbH, Herrsching, Germany) with electrodes placed according to the 10-10 system and with NeurOne 1.3.1.26 software (Mega Electronics Ltd., Kuopio, Finland), and Tesla #MRI 2013011 and #MRI 2013012 amplifiers (Mega

Electronics Ltd.). Additionally, two pairs of bipolar electrodes were used to monitor the electro-oculogram (EOG) and electrocardiogram (ECG).

The level of consciousness was manipulated with either propofol (n=19, one subject withdrew after randomization) or dexmedetomidine (n=20) using TCI with stepwise increasing drug concentrations. Pre-defined concentrations for loss of responsiveness (LOR) from a preceding dose-finding study in the same subjects were used as reference (Kallionpää et al., 2018; Scheinin et al., 2018a). The initial target concentration of the infusion depended on the individually determined concentrations, starting from 0.5 x LOR concentration for each subject, then 0.75 x LOR – 1.0 x LOR until unresponsiveness (UR) was reached. UR was defined as a participant's inability to respond to a standardized pre-recorded responsiveness test (see below). If UR was not reached with 1.0 x LOR, additional 0.25x increments compared to the previous target level were applied at approximately 13 min intervals until UR was reached in every subject. "Moderate sedation" was defined as the last responsive anesthetic level before UR and "light sedation" as the preceding responsive anesthetic level. The concentration needed to induce UR determined the anesthetic level, which was maintained as a pseudo steady-state infusion using TCI for at least 13 min. Then, an attempt was made to arouse the subject with verbal (subject addressed by name) and, if necessary, mild tactile stimuli (a shake in the shoulder). In case of successful recovery to a responsive state (R), structured interviews to probe the subjects' experiences from the UR period were conducted (for details, see "Assessment of the state of consciousness"). The subjects were then left unstimulated and a second UR (UR2) was targeted without adjustment of drug exposure. Thereafter, a second awakening and interview were conducted (R2). Thus, two cycles of different states of consciousness (responsive–unresponsive) were attempted during a constant-rate anesthetic drug infusion. After UR2, or if awakening on the first or second round was unsuccessful, or if a subject did not achieve the UR2 state, the drug concentration was increased by 50 % to achieve a deeper level of anesthesia (1.5 x UR). Finally, the drug infusion was terminated, and the subjects

were allowed to recover. At baseline, at sedative levels and at each achieved state thereafter, brain activity changes reflected by rCBF were measured with repeated PET scans (for details, see "Positron emission tomography imaging").

The behavioral state of the subjects was classified based on a responsiveness test (R-test) that was presented through headphones. The R-test consisted of a pre-recorded set of ten sentences with a semantically congruent (n=5) or incongruent (n=5) last word. The R-test was played at every drug concentration level and whenever another constant-rate UR or R state was targeted. The subjects were instructed to respond by left or right handle-press according to the congruency of the sentence; allocation of hands corresponding to congruous sentences (left or right) was balanced. UR was defined as zero out of ten handle-presses. Each R-test block lasted approximately 90 s, and the same sentence was never repeated. The R-test was presented with the Presentation 17.0 stimulus delivery and experimental control software system (Neurobehavioral Systems Inc., Berkeley, CA, USA). All instructions and stimuli were delivered via headphones. Detailed information regarding stimulus preparation has been described in our previous publication (Kallionpää et al., 2018).

Sleep study (Experiment 2). Thirty-seven subjects from Experiment 1 participated in Experiment 2 (another two subjects withdrew after the anesthesia study). Consumption of alcohol and medications was not allowed in the preceding 48 h and intake of caffeine-containing products was prohibited for 16 h before the study session. The likelihood of falling asleep while inside the PET scanner was increased by requiring sleep deprivation for at least 30 h before the imaging session. Similar EEG equipment as in Experiment 1 was used to record EEG and to monitor sleep stages during the PET scan. For complete polysomnography (PSG), two additional bipolar electrodes were attached on the mentalis and submentalis muscles to record EMG. ECG was monitored as in Experiment 1.

Sleep staging to determine PET scan onsets was done by visual inspection of online PSG by an experienced sleep technician (K.V.) according to The Academy of American Sleep Medicine 2013 (AASM 2013) sleep scoring manual guidelines. The aim was to first scan each subject in the awake state (sleep-deprived wakefulness) and then in as many different sleep stages as possible. The maximum number of scans was restricted to five to avoid excessive radiation exposure. After the first scan during sleep-deprived wakefulness, the subjects were allowed to fall asleep. Once the subject fell asleep, a second PET scan was immediately started (NREM stage N1), followed by a third scan during light sleep (NREM stage N2) and a fourth scan during deep sleep (NREM stage N3). After each scan, the subjects were awakened and interviewed in detail for mental content during the verified sleep stage (for details, see "Assessments of the state of consciousness"). Final sleep staging was conducted offline by two experienced sleep technicians for the 90 s scan time that was used for PET data analysis, applying AASM 2013 guidelines, with an inter-rater agreement of 93.1 % (Cohen's kappa = 0.908, $p<0.001$).

Assessment of the state of consciousness. Maintained responsiveness always indicated a connected state. In both experiments, reports were collected to probe subjective experiences during the preceding unresponsive anesthetic or NREM sleep condition(s). In Experiment 1, the subjects were asked an initial question after each evoked awakening whether dreaming had been present during the unresponsive period (answer options: "yes", "no", "uncertain"). Thereafter, a PET scan was performed to attain an immediate scan from the evoked awakening. A more detailed interview followed, requesting the subjects to report any subjective experiences they might have had during the unresponsive period, including possible awareness of the study surroundings (Radek et al., 2018). In Experiment 2, the detailed interview was conducted immediately after the awakening.

The interviews were digitally recorded and later transcribed word by word for systematic content analysis conducted by two independent judges, to verify disconnectedness during the unresponsive periods. The answer to the initial question in Experiment 1 (yes, no, uncertain) was analyzed to

assess the presence of subjective experiences. In content analysis from both experiments, the judges divided the interview reports into three main categories: 1) reports including no recall of any subjective experiences, 2) white reports, i.e., reports where the participant had a strong impression of experiences during unresponsiveness, but could not recall any specific content, and 3) reports including specific content. The reports including specific content were further categorized as either including internally or externally generated experiences. Internally generated experiences involved hallucinatory contents of consciousness, either dreaming or memory incorporation of the research environment (i.e., experiences related to things/persons that were present or events that had occurred before unresponsiveness ensued), while externally generated experiences referred to awareness of the current environment (experiences related to verifiable stimuli that the participant could not have expected to occur during the experimental session). Reports of no recall of any experiences, white reports, and reports including internally generated experiences were considered to verify disconnectedness, whereas reports of awareness were considered as signs of connectedness during unresponsiveness.

Magnetic resonance imaging (MRI). For each subject, an anatomical brain MRI scan (T1 3D, T2 axial, FLAIR coronal) was obtained before Experiment 1 for subsequent image preprocessing and exclusion of any brain anomalies. A Philips Ingenuity PET-MR 3T scanner (Philips Medical Systems, Best, The Netherlands) was employed. A trained neuroradiologist (MN) evaluated the anatomical images for any pathological findings. Isotropic T1 3D was also used as anatomical reference in PET data analysis.

Positron emission tomography (PET) imaging. PET imaging was performed using an ECAT HRRT brain scanner (Siemens CTI, Knoxville, TN, USA) brain scanner. The HRRT is a dual-layer, LSO-LYSO crystal-detector scanner characterized by a nearly isotropic 2.5 mm intrinsic spatial resolution. In the reconstructed images, spatial resolution varies from 2.5 to 3 mm in the radial and tangential directions and from 2.5 to 3.5 mm in the axial direction in the 10 cm field-of-view

(FOV), and the total length of axial-FOV is 250 mm, covering most of the brain. Subjects were positioned in the scanner in supine position, using a standard headrest and a Velcro band over the forehead to minimize head movements, and head motion was monitored with a high-precision, stereotaxic tracking device (Polaris Vicra, Northern Digital, Waterloo, ON, Canada) attached to the subject's head.

To assess rCBF, $[^{15}O]O_2$ was produced with a low-energy deuteron accelerator Cyclone 3 (IBA, Ion Beam Applications Inc., Louvain-la-Neuve, Belgium) at Turku University Hospital. The target gas with $[^{15}O]O_2$ was mixed with pure H_2 to produce water vapor in a hot (700 °C) quartz furnace. Radiopharmaceutical-grade $[^{15}O]H_2O$ was produced according to GMP using an automated Hidex Radiowater Generator (Hidex Oy, Turku, Finland). A 300 MBq dose of $[^{15}O]H_2O$ was administered in 15 s by an automated infusion system (Rad Injector, Tema Sinergie, Faenza, Italy). Emission data in list-mode format were recorded over the duration of the $[^{15}O]H_2O$ administration and the subsequent 120 s. Point of departure (POD) for emission data was determined offline as the time point where the "trues" count rate exceeded the "randoms" count rate. By default, the list-mode data were histogrammed in two (60 s and 30 s) 3D sinograms from POD onwards. In case the external motion recordings indicated significant (>2.5 mm) within-frame motion, sub-frames were formed until sub-threshold level motion was assured (Johansson et al., 2016). In most cases, sub-framing was not needed; yet, 91 sub-frames in 31 (out of 302) sessions were generated for Experiment 1, and 10 sub-frames in 6 (out of 116) sessions were generated for Experiment 2, and some sub-frames were discarded (in 29 sessions in Experiment 1 and in 2 sessions in Experiment 2) due to shortage of data. There were no marked differences in the number of incidences between the two drugs in Experiment 1. Transmission data acquired just before the first $[^{15}O]H_2O$ administration were used to generate photon attenuation maps, while a single-scatter simulation algorithm was used to estimate the proportion of scattered events and randoms were estimated from the block singles. All corrections were included in an iterative image reconstruction procedure including resolution

313 modelling (PSF-OP-OSEM, 12 iterations, 16 subsets) (Comtat et al., 2008) and motion
314 compensation of the attenuation maps (Johansson et al., 2016). Motion compensated frame-wise
315 data were summed to form a 90 s sum-image for subsequent analysis.

316 **Drug concentration measurements.** Blood samples for drug concentration measurements were
317 drawn into EDTA tubes from a cannulated forearm vein in Experiment 1. Samples were drawn at
318 baseline and at the end of each drug target infusion step. Additionally, a sample was taken in each
319 behavioral state, i.e., whenever the state of consciousness was presumed to have changed.
320 Concentrations of dexmedetomidine in plasma were measured with high-performance liquid
321 chromatography (HPLC) with tandem mass spectrometry. Propofol concentrations were measured
322 with HPLC and fluorescence detection (Yeganeh and Ramzan, 1997).

323 **Neuroimaging data analysis and statistical considerations.** Image pre-processing was performed
324 with standard PET techniques as described above, and an average image of the summed PET
325 images was formed for each condition for each subject. Across subject image alignment,
326 registration and normalization was performed using statistical parametric mapping software
327 (versions 8 and 12, SPM8 and 12; Wellcome Institute, London, UK). A reference frame from the
328 baseline scan was used as a target to obtain initial between sessions realignment and motion
329 correction. The mean PET image was co-registered with the skull-stripped anatomical MRI and the
330 session-images were resliced accordingly into MRI voxel size (1x1x1 mm). Non-linear mapping
331 from the MRI to the MNI standard space was estimated using unified segmentation in SPM8, and
332 the deformations were subsequently applied to the MRI and co-registered PET images. All
333 normalized PET images were smoothed using an isotropic Gaussian kernel of 12 mm FWHM.
334 Proportional scaling was used in the PET analyses.

335 Partial least squares (PLS) software was used to analyze the data for rCBF pattern changes over
336 state transitions. PLS is a multivariate statistical analysis technique that analyses associations

between two sets of data. Here we used PLS to identify brain activity patterns that differ between experimental conditions. The PLS output consists of a set of latent variables (LVs), which are linear combinations of initial variables that maximally co-vary with the corresponding conditions. Statistical significance of each LV was calculated with permutation tests. To assess the reliability of voxels contributing to the LV, bootstrapping was used. The bootstrap ratio is the ratio of the weights to the standard errors estimated from bootstrapping. Therefore, the larger the magnitude of a bootstrap ratio, the larger is the weight (i.e. contribution to the latent variable) and the smaller the standard error (i.e. higher stability) (McIntosh and Lobaugh, 2004; Mišić et al., 2016).

Five thousand permutations were computed to determine the significance of each LV and 5000 bootstrap iterations were run to assess the reliability of identified saliences. Voxels with saliences >2.575 x their standard error (SE), corresponding to an approximate $p<0.01$, were considered statistically significant. All comparisons yielded two LVs of which LV1 explained 100 % of the cross-block covariance and was significant with $p<0.001$, while LV2, representing the residuals, was not significant. All figures are bootstrap ratio figures with thresholds of $p<0.01$ for voxels significantly contributing to the pattern. Since PLS analyzes the data in a multivariate fashion, there is thus only one statistical test and no need to correct for multiple comparisons.

First, we conducted a mean-centered task PLS analysis to establish the patterns of relative blood flow changes between the activity seen in the normal wakeful state (baseline acquired in Experiment 1 for all subjects) and the gradually deepening levels of anesthesia (dexmedetomidine or propofol) and sleep, using separate pairwise analyses. Next, we targeted an analysis to seek for patterns of altered brain activity specifically related to changes in the state of consciousness (connected versus disconnected). We used the same method to analyze state transitions within subjects, between connected and disconnected conditions under light dexmedetomidine or propofol anesthesia and natural sleep, while minimizing the confounding drug and sleep pressure effects. To achieve this, comparisons were now made during constant dose anesthesia or between sleep

deprived baseline and N2 sleep. Successful scans for comparisons were obtained from 19, 14 and 9 subjects in the propofol, dexmedetomidine and sleep subjects ("becoming disconnected) and from 9 and 16 in the propofol and dexmedetomidine subjects ("becoming connected"), respectively. Since only 2 out of 13 previously awakened propofol subjects achieved a second unresponsive state (UR2), we used the condition with least confounding drug effect, i.e. "moderate sedation" *vs.* UR, to examine brain activity changes related to transition from a responsive (and connected) to an unresponsive (and disconnected) conscious state in the propofol group. The final number of successful comparisons between connected and disconnected states was dependent on obtaining both connected and disconnected scans from each subject, and the applied comparisons are clarified in Figure legends 3 and 3-1.

The normality of variables was checked using the Shapiro-Wilk test. Fisher's exact test was used to compare arousability and responsiveness between the treatments. Paired and unpaired t-tests were used to compare measured drug concentrations between the disconnected and connected conditions.

Results

Realization of experimental designs. All of the targeted states, interviews and scans were not obtained in every subject. In Experiment 1, 13 out of 19 propofol subjects (68 %) and 16 out of 20 dexmedetomidine subjects (80 %) were arousable during the fixed-dose drug infusion (Fisher's exact test, $p=0.480$, $df=1$). No significant within subject differences were observed in drug concentrations between the fixed-dose responsive and fixed-dose unresponsive states ($p>0.2$ for both drugs, Table 2). The measured drug concentrations were higher in those subjects who were not arousable in both drug groups ($p<0.05$ for both). The numbers of successful rCBF PET scans were [n=propofol (obtained from % subjects), n=dexmedetomidine (%)]: wakeful baseline [n=19 (100 %), n=20 (100 %)], light sedation [n=14 (74 %), n=6 (30 %)], moderate sedation [n=19 (100 %), n=20 (100 %)], UR [n=19 (100 %), n=20 (100 %)], R [n=9 (47 %), n=16 (80 %)], UR2 [n=2 (11 %), n=15 (75 %)], R2 [n=2 (11 %), n=14 (70 %)], 1.5 x UR [n=15 (79 %), n=16 (80 %)]. Four awakened propofol subjects could not be scanned in the R-state because of fluctuations in behavior (3 subjects) or intravenous line malfunction (1 subject). Within-subject pairs of images were used in the connected/disconnected analysis and hence, the number of comparisons in this analysis may be different from the total number of obtained scans.

In Experiment 2, sleep-deprived baseline scans (awake) were not obtained from all subjects because of inability to remain awake during the scan. Altogether, 32 subjects fell asleep at least once (86 %). While some subjects reached the same sleep stage and were awakened from it several times, only the first successful scan obtained from each achieved sleep stage was used. The numbers of first successful rCBF PET scans were [n= state, (achieved by % of subjects)]: Sleep deprived wakefulness [n=22 (59 %)], N1 [n=14 (38 %], N2 [n=24 (65 %)], N3 [n=14 (38 %)]. Within-subject pairs of images were used in the connected/disconnected analysis and hence, the number of comparisons in this analysis may be different from the total number of obtained scans.

In those subjects who could be interviewed, subjective experiences (comprised of white reports and reports including specific content) were reported in 80 % and 71 % of the interviews in Experiments 1 and 2, respectively. Most often, internally generated dreaming or memory incorporation were described. In Experiment 1, the recall rates of subjective experiences were equal (80 % of interviews) in both drug groups. In Experiment 2, subjective experiences were reported in 58 %, 66 %, and 83 % of the N1, N2 and N3 interviews, respectively.

Signs of awareness were reported by one subject receiving propofol and one subject receiving dexmedetomidine, both after the second unresponsive period in Experiment 1, and by one subject after N2 sleep in Experiment 2. The scans obtained from these states were not considered to represent a disconnected state, and were excluded from the connected vs. disconnected comparisons. Apart from these cases, unresponsiveness denoted disconnected, albeit mostly not unconscious, states.

Separation of changes in brain activity related specifically to consciousness from the overall effects of anesthesia. In Experiment 1, we scanned 39 healthy subjects with PET in multiple conditions varying in terms of administered anesthetic agent, the level of drug exposure and the subjects' responsiveness. All drug concentration levels and behavioral states were first compared to an awake baseline without drug to reveal overall effects of the drugs on brain activity. We discovered that both drugs similarly suppressed rCBF. The most profound reductions were seen in frontal, parietal and temporal cortical regions and subcortically mainly in the thalamus, whereas primary sensory and motor cortices were less affected (Fig. 2A). The portrayed effects were not indicative of behavior (responsiveness) or state of consciousness as they were already evident at sedative drug concentrations. They thus depicted the combined influences of the drug(s) and the state of consciousness.

To unmask the confounding pharmacologic effects, we utilized the forced awakening paradigm during constant-rate anesthetic drug infusions, and compared changes in brain activity between connected and disconnected states of consciousness at similar measured drug concentrations (Table 2). Most subjects were arousable during the fixed-dose infusions and 80 % of the arousable subjects reported subjective experiences in the immediate interview. Thus, the subjects were mostly in a disconnected and conscious, rather than an unconscious, state. Within-subject comparisons were made between connected and disconnected states, where the concentration-dependent drug effects on the brain were controlled by the study design. We thereby explored i) which functional changes best associate with loss and return of connected consciousness and ii) whether the transitions between these two states of consciousness are reciprocal and symmetrical. We discovered that the activity of a restricted network of core midline brain structures including the thalamus, anterior cingulate cortex (ACC), posterior cingulate cortex (PCC) and the angular gyri in the inferior parietal lobules was consistently associated with the connected state (Fig. 3A and Figs. 3-2–3-5). This network was activated and deactivated in an opposite (reversed) manner, independently of the drug administered. The more extensive suppression of frontoparietal cortical areas that was seen in comparisons against no-drug awake baseline neither manifested during transition to a disconnected state, nor an analogous activation of these areas was seen at recovery to a connected state. Some cortical effects were observed, but they were heterogeneous in terms of direction of change, drug, and areas affected (Fig. 3-1A). Consistent state-specific differences in brain activity were witnessed only within a restricted network of midline structures and the angular gyri.

Physiological sleep resembles anesthesia. The same subjects (n=37 due to two withdrawals) participated in the sleep experiment, where no pharmacologic interventions were used to manipulate consciousness. Compared to awake baseline (acquired in Experiment 1), the suppression of rCBF during sleep deprivation and N1, N2 and N3 sleep resembled the effects of increasing anesthetic exposure (Fig. 2B). The largest suppression of blood flow was observed in the higher-order frontal,

447 parietal and temporal cortical regions and in some subcortical structures, such as the thalamus,
448 whereas activity was relatively preserved in lower-order somatosensory and motor cortical regions.
449 Overall, physiological sleep seemed to suppress blood flow similarly to the two different anesthetic
450 agents.

451 Next, we tested whether the effect of strong sleep pressure and resulting drowsiness due to sleep
452 deprivation could be minimized by comparing the sleep-deprived (connected) state to N2 sleep
453 (disconnected state). Overall, N2 sleep was followed by a report with subjective experiences in 66
454 % of the immediate interviews. Thus, most subjects were in a disconnected, rather than a fully
455 unconscious state. Within-subject comparisons were made between connected and disconnected
456 states, aiming to reveal which functional changes associate best with loss of connected
457 consciousness. The results were clearly distinct from those of the first analysis. A restricted network
458 of core midline structures including the thalamus, anterior and posterior cingulate cortices, bilateral
459 angular gyri, dorsolateral prefrontal cortex and right caudate nucleus was consistently associated
460 with the state of consciousness (Fig. 3B and Fig. 3-6). Cortical renderings showed that a sleep-
461 induced change in the state of consciousness was accompanied with only minimal activity changes
462 on the cortical surface (Fig. 3-1B).

Discussion

It has been widely accepted that a broad network of frontoparietal cortical areas contribute to consciousness and its contents (Baars et al., 2003). It is also indisputable that brain activity is globally reduced and that communication between different brain areas is disrupted during different unconscious states. The activity of distinct medial or lateral subsystems has been implicated in awareness, specifically awareness of self and the environment, respectively (Boly et al., 2008). It is not equally well characterized, what specifically accounts for loss or recovery of a connected state, and what the necessary preceding effects are that enable this transition. The complexity of these phenomena in the brain and the diverse experimental designs across studies have complicated the forming of a unified view on the neural correlates and mechanisms of consciousness, resulting in an understandable rivalry between different theories (Reardon, 2019).

We used an established PET method to monitor brain activity and employed novel designs to overcome some past limitations related to experimental studies on human consciousness. Two experiments targeting unresponsive anesthetic states and physiological sleep revealed that the induced conditions represented mostly disconnected, rather than unconscious, states. We discovered that connected and disconnected states of consciousness were best differentiated by activity in the core midline structures of the brain, including the thalamus, cingulate cortices and angular gyri. Only minimal and inconsistent differences on the cortical surface were witnessed between these two conditions, suggesting a lesser contribution of the outer cortex to the connected state *per se*.

In previous studies using anesthesia and sleep, distinct patterns of altered brain activity and/or connectivity during unresponsive states have been described (Boveroux et al., 2010; Liu et al., 2013; Akeju et al., 2014; Ranft et al., 2016; Warnaby et al., 2017; Braun et al., 1997; Kajimura et al., 1999). It has been shown that information transfer across frontoparietal cortical areas is disrupted during sleep and anesthesia (Massimini et al., 2005; Boly et al., 2012), but there is also

strong evidence to support that thalamic activity is more crucial and critically involved in cortical regulation (Alkire et al., 2000; Xie et al., 2011; Långsjö et al., 2012; Baker et al., 2014). Involvement of the insula (Warnaby et al., 2016) and angular gyri (Legostaeva et al., 2019) have also been demonstrated. Anesthesia and sleep have also both been shown to disrupt thalamic connectivity to the higher-order cortex (Akeju et al., 2014; Guldenmund et al., 2017), while lower-order sensory circuits are less impacted (Boveroux et al., 2010; Liu et al., 2013), experimentally supporting 'cognitive unbinding' as mechanistic for unconsciousness (Mashour, 2013). Our current findings neither contradict any previous work, nor do we question the suppression of any previously described neuronal circuit in conjunction with anesthesia or sleep. We highlight, however, the importance of relevant comparisons in experimental studies on consciousness; all changes in brain activity do not exclusively reflect changes in the state of consciousness. Indeed, we were able to partly overcome confounding effects by choosing the most relevant scan as the wakeful reference. With our approach, a shift between connected and disconnected states associated best with the changes within a restricted network of midline structures and the angular gyri. Interestingly, the method used to manipulate consciousness, i.e., anesthetic agent or physiological sleep, seemed to have minimal effect on the results.

Our findings suggest that widespread cortical suppression is not sufficient, albeit perhaps necessary, for loss of connected consciousness. In our previous study (Långsjö et al., 2012), awakening during constant-dose dexmedetomidine administration was associated with activation of the anterior cingulate cortex, thalamus and the brainstem, i.e., phylogenetically old cortical regions and the arousal system. The emerged regions overlap with distinct neuronal networks implicated in human consciousness: The default mode network (DMN) is considered to be foundational for self-referential mentation whereas the executive control network (ECN) for externally guided awareness. The salience network (SN) is thought to play a role in coordinating between the DMN and ECN (Demertzi et al., 2013; Menon&Uddin, 2010). Unresponsive states of different etiologies

512 have shown to associate with suppression or disruption of functional connectivity within these
513 networks (Guldenmund et al., 2017; Boveroux et al., 2010; Qin et al., 2015; Huang et al., 2020),
514 corroborated by the findings of the current study. Interestingly, decreased rCBF or blood oxygen
515 level dependent (BOLD) fMRI signal in DMN areas and thalamus can also be seen in a psychedelic
516 state induced by psilocybin (Carhart-Harris et al., 2012) and in DMN areas during meditation
517 (Brewer et al., 2011). Both psychedelic and meditative states have been associated with decreased
518 sense of self. Indeed, general anesthesia has been characterized as 'fragmentation of selfhood'
519 (Sleigh et. al., 2018). We are tempted to speculate that while the global state seemed most reliant on
520 the activity of the thalamus, the DMN and the SN, a disconnected state also needs a preceding
521 deactivation of the cortex.

522 Surprisingly identical effects were induced by the different interventions, despite the distinct
523 molecular mechanisms of action of the two drugs and the complex cascades of sleep regulation
524 (Saper et al., 2005). Our findings suggest a partly unitary neural mechanism to operate behind the
525 investigated conditions. Indeed, dexmedetomidine has been suggested to induce a state resembling
526 physiological sleep, as assessed by both behavioral and electrophysiological features (Nelson et al.,
527 2003; Huupponen et al., 2008), whereas propofol is considered different in this respect.
528 Interestingly, forced awakening turned out to be feasible also in most of the propofol subjects, and
529 this quality may be exploited in experimental studies on consciousness.

530 When relating our findings to theories of the neural mechanisms of consciousness, the distinction
531 between the state of being conscious *vs*. the specific contents of consciousness becomes relevant.
532 As to the contents of consciousness, most theories place the neural correlates of consciousness to
533 particular cortical networks: the Neural Global Workspace model (Dehaene and Changeux, 2011) to
534 long-range frontoparietal connections, the Recurrent Processing theory (Lamme, 2010) to local
535 recurrent activities in the ventral occipitotemporal cortex and the Posterior Hot Zone model (Koch
536 et al., 2016) to posterior cortical areas, excluding the frontal cortex. As to the state of being

conscious, which is necessary for any contents of consciousness to manifest, most theories (Llinás et al., 1998; Tononi and Edelman, 1998; Koch et al., 2016) emphasize subcortical and thalamocortical connections. Our findings reveal the necessary and minimally sufficient mechanisms for the connected state, supporting and refining the latter theories. As we did not investigate any specific contents of consciousness, our results cannot resolve the current controversy between the posterior *vs.* frontoparietal theories of the contents of consciousness. However, our results clearly show that frontal and frontoparietal cortical areas were strongly affected already before a disconnected state was reached. Thus, they (or indeed any superficial cortical areas) do not seem to be necessary for the connected state as such, even though they may be necessary for particular contents of consciousness.

Important methodological limitations related to the present study needs to be addressed. Verification of the disconnected state was based on the subjects' responsiveness and reports of mental content, neither of which can indisputably verify a persons' actual tate of consciousness. A motor response to a presented stimulus is dependent on the type and salience of the chosen stimulus, as well as the complexity of the requested behavioral output. The superiority of any stimulus has not, to our knowledge, been characterized. Retrospective subjective reports, in contrast, are strongly dependent on memory. Internal conscious experiences are commonly reported after experimental and clinical anesthesia (Sanders et al., 2012; Cascella et al., 2015; Gyulaházi et al., 2016; Radek et al., 2018) and upon awakening from all stages of sleep (Nielsen, 2000). However, the lack of a dream report does not unequivocally indicate unconsciousness (Windt et al., 2016), and the lack of an awareness report after anesthesia does not necessarily prove disconnectedness (Sanders et al., 2017). Especially delayed interviews must be considered unreliable because of amnesia caused by anesthetics or sleep (Schwartz and Maquet, 2002; Hudetz, 2008). Retrospective reports remain, however, the only way to access subjective experiences during an unresponsive state. In a recent study on dreaming, a similar awakening paradigm with immediate

562 interviews was utilized. Based on individual EEG patterns, it was possible to predict with 87 %
563 total prediction accuracy across all states whether a subsequent report from NREM and REM sleep
564 included dreaming (Siclari et al., 2017), providing important validation of the report-based state
565 classification.

566 Our study had several strengths: We employed identical dosing schemes for two very different
567 anesthetic drugs resulting in similar behavioral end-points. The same subjects participated in the
568 subsequent sleep study. This enabled cross-study and within-subject comparisons using identical
569 data acquisition and analysis procedures. We identified a network of core brain structures where
570 activity consistently associated with the state of consciousness (connected or disconnected).
571 Anesthesia and sleep had state-specific effects that were distinct, reciprocal and separable from
572 their overall effects on brain activity. Stringent and accurate definitions of the explored states and
573 their proper comparisons are of vital importance, as anesthetic-induced unresponsiveness and sleep
574 rarely provide complete unconsciousness.
575

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740 **Figure Legends**

741 **Figure 1. Design of Experiments 1 and 2**. Behavioral states of interest in the anesthesia study: W
742 = wakeful baseline, SED_{light} = light sedation, SED_{mod} = moderate sedation state, UR = unresponsive
743 state, R = responsive state, UR2 = second unresponsive state, R2 = second responsive state, 1.5 x
744 UR = unresponsiveness with 1.5 x UR anesthetic dose. Note the fixed-dose anesthetic level (UR
745 dose) with steady-state infusion in UR, R, UR2 and R2. For details, see Material and Methods.

746 Behavioral states of interest in the sleep study: SDW = sleep deprived wakefulness, N1, N2, N3 =
747 NREM sleep stages N1, N2 and N3. For details, see Material and Methods.

748 **Figure 2. Relative rCBF suppression at different anesthetic levels, sleep stages and behavioral**
749 **states.** Images showing the global pattern of rCBF changes in association with (A) different levels
750 of propofol or dexmedetomidine and (B) different sleep stages. All states are compared to a non-
751 sleep-deprived awake baseline with no drug. Cool colors show the largest and warm colors the
752 smallest relative suppression ($p<0.01$; color bars depict bootstrap ratios in PLS). Light and
753 moderate sedation indicate responsive levels during escalating drug exposure. Unresponsive (UR)
754 dose refers to drug concentration titrated individually to induce unresponsiveness, and 1.5 x UR
755 dose refers to 50 % higher doses. The states of consciousness (connected or disconnected) during
756 unresponsive UR and 1.5 x UR levels could not be verified because of lack of immediate interviews
757 in unarousable subjects and/or after terminating the infusion, and are therefore marked as
758 "(disconnected?)". Maximal suppression is seen in frontal and parietal cortical areas, as well as in
759 subcortical structures, and the pattern is evident already during light sedation, resembling the awake
760 sleep-deprived state. The intensity of suppression increases with drug dose level and depth of sleep,
761 regardless of the behavioral state.

762 Light Sedation (SED_{light}, propofol: n=14, dexmedetomidine: n=6), Moderate Sedation (SED_{mod},
763 propofol: n=19, dexmedetomidine: n=20), UR Dose and unresponsive (UR, propofol: n=19,

764 dexmedetomidine: n=20), UR Dose and responsive = forced awakening during anesthetic infusion
765 (R, propofol: n=9, dexmedetomidine: n=16), 1.5 x UR Dose (propofol: n=15, dexmedetomidine:
766 n=16); SDW = sleep-deprived wakefulness (n=22), N1, N2, N3 = NREM sleep stages N1 (n=14),
767 N2 (n=24) and N3 (n=14); all targeted states were not achieved in all subjects.

768 **Figure 3. Differences in relative rCBF between connected and disconnected states of**
769 **consciousness.** A central core network of consciousness was revealed by imaging anesthetic- and
770 sleep-induced state transitions. Cool colors show the largest and warm colors the smallest relative
771 suppression upon becoming disconnected (left panel) and warm colors show the largest and cool
772 colors the smallest relative activation upon becoming connected (right panel) ($p<0.01$, corrected;
773 color bars depict bootstrap ratios in PLS). A) During infusions of both propofol (upper panel) and
774 dexmedetomidine (middle panel), state-specific analyses between connected and disconnected
775 conditions revealed that a network of core midline structures was activated and deactivated in a
776 reciprocal manner, with minimal effects seen on the cortical surface. Activity of the thalamus,
777 anterior and posterior cingulate cortices, precuneal area and bilateral angular gyri showed the most
778 consistent associations with the subjects' state of consciousness. B) During physiological sleep
779 (lower panel), transition from sleep-deprived wakefulness to N2 sleep revealed the deactivation of
780 the same core structures. Again, changes in cortical surfaces were inconsistent. Brain regions with
781 statistically significant differences are listed in Figures 3-2–3-6, and cortical renderings are shown
782 in Figure 3-1 (extended data).

783 ACC = anterior cingulate cortex, AG = angular gyrus, dMPFC = dorsomedial prefrontal cortex,
784 PCC = posterior cingulate cortex, pCUN = precuneus, PHG = parahippocampal gyrus, vMPFC =
785 ventromedial prefrontal cortex. Successful scans for within-subject comparisons were compared in
786 19 (SED_{mod}→UR), 14 (R→UR2) and 9 (SDW→N2) propofol, dexmedetomidine and sleep subjects
787 (left panel: connected → disconnected) and in 9 (UR→R) and 16 (UR→R) propofol and
788 dexmedetomidine subjects (right panel: disconnected → connected), respectively.

Table Legends

Table 1. Cognitive and behavioral characteristics of connected consciousness, disconnected consciousness and unconsciousness.

Table 2. Targeted and measured drug concentrations during Experiment 1.

Extended Data Figure Legends

Figure 3-1. Differences in relative rCBF on the cortical surface between connected and disconnected states of consciousness. Cortical renderings illustrating state-related changes in brain activity revealed by imaging anesthetic- and sleep-induced state transitions. Cool colors show the most and warm colors the least relative suppression upon becoming disconnected (1st, 3rd and 5th rows), and warm colors the most and cool colors the least relative activation upon becoming connected (2nd and 4th rows) ($p<0.01$, corrected; color bar depicts bootstrap ratios in PLS). The figure illustrates minimal cortical effects, and they were heterogeneous in terms of direction of change, drug, and areas affected. For subcortical renderings, see Figure 3.

Successful scans for within-subject comparisons were compared in 19 (SED_{mod}→UR), 14 (R→UR2) and 9 (SDW→N2) propofol, dexmedetomidine and sleep subjects (connected → disconnected, rows 1, 3 and 5) and in 9 (UR→R) and 16 (UR→R) propofol and dexmedetomidine subjects (disconnected → connected, rows 2 and 4), respectively.

Tables

Table 1. Cognitive and behavioral characteristics of connected consciousness, disconnected consciousness and unconsciousness.

		Disconnectedness	
	Connected Consciousness	Disconnected Consciousness	Unconsciousness
Awareness of external stimuli	Yes	No	No
Behavioral responsiveness	Yes*	No	No
Subjective experiences	Yes	Yes	No

* Responsiveness may be absent in rare cases such as the locked-in syndrome or during muscle paralysis in conjunction with unsuccessful general anesthesia. Modified from Sanders et al., 2012; Bonhomme et al., 2019

Table 2. Targeted and measured drug concentrations during Experiment 1.

Drug	Light Sedation		Moderate Sedation		UR Dose / Disconnected		UR Dose / Connected		1.5x UR Dose		Recovery	
	Targeted	Measured	Targeted	Measured	Targeted	Measured	Targeted	Measured	Targeted	Measured	Estimated	Measured
All subjects												
Propofol (µg/ml)	1.13 (0.37) n=13	0.73 (0.39)	1.37 (0.49) n=18	1.01 (0.51)	1.78 (0.56) n=18	1.48 (0.60)	1.47 (0.42) n=8	1.13 (0.31)	2.74 (0.81) n=15	2.46 (0.77)	1.14 (0.37) n=15	1.16 (0.35)
Dexmedetomidine (ng/ml)	1.19 (0.38) n=6	0.98 (0.54)	1.06 (0.54) n=20	1.10 (0.58)	1.50 (0.56) n=20	1.80 (0.66)	1.24 (0.33) n=16	1.54 (0.37)	2.38 (1.05) n=16	3.27 (1.32)	1.38 (0.51) n=17	1.60 (0.64)
Subjects who could be awakened during constant infusion												
Propofol (µg/ml)					1.47 (0.42) n=8	1.06 (0.25)	1.47 (0.42) n=8	1.13 (0.31)*				
Dexmedetomidine (ng/ml)					1.24 (0.33) n=16	1.48 (0.40)	1.24 (0.33) n=16	1.54 (0.37)§				

Mean (SD) targeted or estimated and measured drug concentrations in plasma during light and moderate (last responsive anesthetic level before losing responsiveness) sedation, disconnected and connected states of consciousness during constant infusion titrated to unresponsiveness (UR Dose / Disconnected and UR Dose / Connected, respectively), deep unresponsive state (1.5x UR Dose) and responsive state after terminating the drug infusion (Recovery) in the propofol (n=19) and dexmedetomidine (n=20) groups. No statistically significant differences in the measured concentrations between the disconnected and connected states in subjects who could be awakened (*p=0.880, df=7; §p=0.203, df=15; paired t-tests after Bonferroni correction). The numbers vary because not all states were achieved in every subject and because of few missing blood samples.

(Extended data in two separate files: Figure 3-1 as one tiff file and Figures 3-2, 3-3, 3-4, 3-5 and 3-6 in one Word file)