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_____ TITLE PAGE:

_____ Brief Title (Ideally less than 120 characters)

Anesthesia-induced suppression of human dorsal anterior insula responsivity at loss of volitional behavioral response

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26 Anterior insula suppression and responsiveness
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34 **_____ Brief (1-3 sentence) summary statement for the table of contents, where**
35 **applicable**

36 It is unclear which brain regions govern loss of behavioral responsiveness (LOBR) during
37 general anesthesia. Here, we show that activity in response to multisensory stimuli is
38 suppressed within a region of the right anterior insula cortex at the point where
39 subjects lose volitional response during an ultraslow induction of anesthesia with
40 propofol. Suppression of the dorsal anterior insula cortex (dAIC) is associated with
41 reductions in fMRI functional connectivity and EEG synchrony in frontoparietal brain
42 regions around LOBR.
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1 Conflicts of interest

2 Patent applications were filed by Isis Innovation, the technology transfer company of
3 the University of Oxford, on perception loss detection. All authors (except JS and MS)
4 are listed as inventors. The authors declare no competing interests.

ABSTRACT

2

Background

It has been postulated that a small cortical region could be responsible for the loss of behavioral responsiveness (LOBR) during general anesthesia. We hypothesize that any brain region demonstrating reduced activation to multisensory external stimuli around LOBR represents a key cortical gate underlying this transition. Furthermore, we hypothesize that this localized suppression is associated with breakdown in frontoparietal communication.

10

Methods

During both simultaneous electroencephalography and functional magnetic resonance imaging (EEG-fMRI) and EEG data acquisition, 15 healthy volunteers experienced an ultraslow induction with propofol anesthesia while a paradigm of multisensory stimulation (i.e. auditory tones, words, and noxious pain stimuli) was presented. We performed separate analyses to identify changes in 1) stimulus-evoked activity, 2) functional connectivity and 3) frontoparietal synchrony associated with LOBR.

18

Results

Using an fMRI conjunction analysis, we demonstrated that stimulus-evoked activity was suppressed in the right dorsal anterior insula cortex (dAIC) to all sensory modalities around LOBR. Furthermore, we found that the dAIC had reduced functional connectivity with frontoparietal regions, specifically the dorsolateral prefrontal cortex and inferior parietal lobule, after LOBR. Finally, reductions in the EEG power synchrony between

1 electrodes located in these frontoparietal regions was observed in the same subjects
2 after LOBR.

3

4 **_____ Conclusions**

5 We conclude that the dAIC is a potential cortical gate responsible for LOBR. Suppression
6 of dAIC activity around LOBR was associated with disruption in frontoparietal networks
7 that was measurable using both EEG synchrony and FMRI connectivity analyses.

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1 _____ TEXT:

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3 _____ Introduction

4 Understanding how the brain generates and maintains consciousness is a major
5 challenge in clinical neuroscience. Delivery of anesthesia during functional
6 neuroimaging therefore offers a powerful tool to investigate the neurobiology
7 underlying consciousness. As anesthetic drug dose is increased, individuals transfer
8 through a continuum of states from alert wakefulness to deep unconsciousness. A key
9 feature in this continuum is loss of behavioral responsiveness (LOBR) to stimulation,
10 where individuals are no longer willing or able to interact with their external
11 environment. Loss of responsiveness is often used to define anesthesia-induced loss of
12 consciousness in both clinical and experimental settings¹, primarily due to the fact that
13 this behavioral endpoint is often the only outwardly observable metric.

14

15 The advent of functional neuroimaging of anesthesia, in addition to the vast historical
16 supporting evidence, has highlighted the fact that loss of responsiveness and loss of
17 consciousness are not necessarily equivalent states (see ² for a review). Neuroimaging
18 of the anesthetized *resting* brain has produced conflicting evidence with regards to the
19 sequence and degree of network connectivity disruption in the large-scale thalamo-
20 cortical and cortico-cortical networks observed around LOBR and deeper levels of
21 anesthesia³⁻⁸. Recent work from our laboratory exploring stimulus-evoked rather than
22 resting changes using simultaneous electroencephalography and functional magnetic
23 resonance imaging (EEG-fMRI) has demonstrated that thalamocortical processing of

multisensory stimuli persists past the point of LOBR up until slow wave activity saturation⁹.

It has been postulated previously that a small cortical region could underpin loss of behavioral responsiveness under anesthesia^{1,2}. However, no study has yet explicitly shown what brain regions govern this earlier and clinically measurable loss of behavioral response. Identifying the brain regions responsible for LOBR is crucial to understanding the subsequent changes in brain function observed at higher doses of general anesthesia, and has important implications for treating patients with disorders of consciousness.

Here, we present three analyses targeted to identify changes in neural processing specifically associated with the LOBR transition under propofol anesthesia. Firstly, we present a whole brain fMRI analysis to identify stimulus-evoked changes to laser pain, auditory tones and word stimuli around loss of responsiveness. We hypothesize that any brain regions demonstrating reduced activity across the LOBR transition to all of the *multisensory* external stimuli presented will represent key brain regions underlying loss of volitional behavioral responsiveness under general anesthesia.

Having identified the dorsal anterior insula cortex (dAIC) *a posteriori* as a potential cortical gate that underlies LOBR, we hypothesize that due to its extensive structural and functional connectivity^{10,11} suppression of dAIC activity could act as the seed for the subsequent breakdown of frontoparietal feedback^{6,12} and loss of information integration^{13,14} observed at deeper levels of anesthesia. Therefore, we present a whole-

1 brain FMRI analysis to identify changes in the functional connectivity of the dAIC around
2 LOBR. Finally, we demonstrate that these reductions in the functional connectivity of
3 the dAIC can be observed as reductions of EEG synchrony between frontoparietal
4 electrodes, corresponding to the dorsolateral prefrontal cortex and inferior parietal
5 lobule regions, identified by the FMRI connectivity analysis.

6

7

Materials & Methods

Overview

Healthy volunteers participated in two neuroimaging experiments; the first experiment was a laboratory-based electroencephalography (EEG) study and the second was a simultaneous EEG-fMRI study. Both studies were approved by the Local Research Ethics Committee (Oxford Research Ethics Committee B, Oxford, UK) and performed in the same subjects between October and December 2009. The same experimental paradigm was used for both studies and consisted of data acquisition in four phases (Fig. 1A). The volunteers experienced a resting period with eyes closed and no drug administration for 10 minutes (phase 1), followed by an ultraslow induction to loss of consciousness using propofol sedation (phase 2). A target-controlled intravenous infusion of propofol was used with step increases of 0.2µg/ml to achieve a maximum effect site concentration (ESC) of 4µg/ml over 48 mins. After resting at the peak propofol dose for 10 minutes (phase 3), the propofol sedation was switched off and subjects were allowed to emerge naturally from unconsciousness over 48 minutes (phase 4).

Noxious laser stimuli, computer generated tones and auditory word tasks were presented to the subjects during the induction and emergence phases (i.e. phases 2 and 4). Loss of appropriate motor response to the cognitive auditory word task was used to assess loss of behavioral response (LOBR) in the healthy volunteers. No behavioral response was sought after the laser or tone stimuli. The data presented here correspond to the stimulus-evoked fMRI responses collected during the induction to

1 loss of consciousness (phase 2) of the simultaneous EEG-FMRI study. This is a targeted
2 analysis of the imaging data previously presented⁹.

3

4 **Sensory stimulation**

5 Three blocks of sensory stimulation were delivered using Presentation software
6 (www.neurobs.com) during the induction to loss of consciousness phase. Each block of
7 16 minutes duration contained interleaved stimuli presented in a pseudorandomised
8 order with approximately one word task, one computer-generated tone and two
9 noxious laser stimuli per minute. The mean inter-stimulus interval (ISI) and standard
10 deviation (SD) for all stimuli was 15 ± 2 seconds (range 12-19 seconds).

11
12 All auditory stimuli (tones and word tasks) were presented binaurally using magnetic
13 resonance compatible electrostatic headphones (MRC Institute of Hearing Research,
14 Nottingham). Auditory volume adequacy was checked before each experiment and
15 while the scanner was running by playing sample stimuli.

16
17 *Auditory word discrimination task:* The word discrimination task was based on the task
18 used by Gaab and coworkers¹⁵. Subjects were presented with pairs of words and then
19 asked to perform a simple decision-making task by responding, using a two-option
20 button box, whether the words were the SAME or DIFFERENT. The word pairs were
21 presented with mean inter-stimulus interval (ISI) of 56.8 seconds (range 16-104s). Each
22 stimulation block contained 8 SAME and 8 DIFFERENT word pairs. The words were
23 selected at random from a list of 200 single syllable words from the MRC
24 Psycholinguistics Database (Machine Usable Dictionary v2.0). These words had a

familiarity of 488 ± 99 (mean \pm SD) and concreteness of 438 ± 120 (mean \pm SD) and were recorded by a male actor using Audacity software (<http://audacity.sourceforge.net>).

Once a word stimulus had been presented it was not used again.

Tones: Computer generated tones (1kHz, 60 ms) were presented with a mean ISI of 60s (range 16–92s).

Noxious laser stimuli: Brief laser stimuli (5ms duration) were applied to provide an acute noxious stimulus that selectively activated the A-delta and C nerve fibres. Nd:YAP laser stimuli (STIMUL 1340, Elen Engineering, Italy) of wavelength $1.34\mu\text{m}$ were applied to the right lower leg with a mean ISI of 30s (range 13–48s) at a subjective intensity rating of 5/10. The energy required to achieve this subjective rating (anchored by 0 = no sensation, 1 = just painful and 10 = most intense sensation) was established in a thresholding session prior to scanning. To avoid skin damage and nociceptor sensitization or habituation, the site of stimulation was moved after each laser stimulus within a marked 6cm^2 area. For both experiments, the subject and investigators wore protective goggles. Numerical ratings for pain intensity were sought after the laser stimulation during the laboratory EEG session only.

Propofol delivery and physiological monitoring

A 1% propofol solution (Diprivan, AstraZeneca UK Limited) was administered intravenously in the left forearm using a pump (Alaris Asena PK, Cardinal Health, UK) and target-controlled infusion (TCI) module (Diprifusor, AstraZeneca UK Limited, UK). This incorporates the Marsh pharmacokinetic model and a pharmacodynamic delay

1 derived from behavioral and EEG parameters. During the propofol-induced loss of
2 consciousness in phase 2, the target ESC was increased by 0.2µg/ml every two minutes
3 for 38 minutes and then continued for a further 10 minutes while the ESC attained the
4 maximum target dose of 4.0µg/ml. The target ESC of 4.0µg/ml produced deep sedation
5 in all volunteers, even accounting for the inter-individual variation in these
6 pharmacodynamic and pharmacokinetic parameters.

7
8 An anesthesiologist was responsible for the administration of propofol and subject
9 physiological monitoring. The anesthesiologist did not have any other study
10 responsibilities and stopped the experiment if they had any concerns over the safety of
11 the volunteer. Oxygen saturation, electrocardiography (ECG), respiratory rate and depth
12 were recorded electronically (MP150, BIOPAC, California, USA). Subjects wore nasal
13 cannulae that allowed simultaneous delivery of oxygen at 2 litres/min and capnographic
14 measurement of expired carbon dioxide was performed (GasAnalyser module, BIOPAC,
15 California, USA). Non-invasive blood pressure was recorded manually.

16 17 **Participants**

18 Healthy volunteers between the ages of 18 and 50 years were recruited by local
19 advertising. All participants gave written informed consent and received financial
20 compensation for study participation. Subjects were medically screened during an initial
21 visit to ensure they met the American Society of Anesthesiologists (ASA) physical status
22 grade I or II and were not susceptible to airway obstruction. Potential participants were
23 excluded for history of tobacco or illicit drug use, high alcohol intake (>14units/week),

1 allergy to anesthetic drugs, difficult venous access, contraindications to MRI and
2 psychiatric, neurological or psychological pathology.
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1 Participating volunteers were given written instructions that followed the day case
2 anesthesia guidelines from the Association of Anaesthetists of Great Britain and Ireland
3 (AAGBI) specifying fasting, travel and supervision standards required to ensure their
4 safe participation. Experiments were carried out in the morning to minimize the
5 discomfort of fasting.

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10 As there is limited literature on stimulus-evoked BOLD imaging during anesthesia, at this
11 stage the number of participants was selected based on our publications and prior
12 experience, alongside this literature ^{6,16–19}. We chose a subject number equal to or in
13 excess of these reported studies in order to provide a sufficient sample size to test our
14 hypothesis. Consequently, we recruited 16 volunteers into the study initially.

16 **MRI data acquisition**

17 Whole brain blood-oxygen level dependent (BOLD) fMRI images (resolution
18 3x3x3.5mm, TR= 3s) were obtained using an echo-planar imaging (EPI) sequence on a 3T
19 MRI scanner as previously reported⁹. Each induction scan consisted of 964 volumes
20 (including four dummy volumes). High-resolution 1 mm³ T1-weighted anatomical scans
21 were also acquired for co-registering the individual volunteer scans to standard
22 stereotactic space. During MRI data acquisition, cushions were used to support the
23 subjects' head and neck, maintaining a hands-free "jaw-thrust" maneuver to ensure
24 airway patency when in the supine position and also minimizing head movement.

1

2 **EEG data acquisition**

3 Large artefacts are induced in the EEG data by the fMRI gradients and ballisto-
4 cardiographic effects during data acquisition inside the scanner^{20,21}. As we were
5 interested in subtle changes in spatial correlations based on the fluctuations in the
6 power spectrum, we analyzed the artifact-free EEG data that had been collected in the
7 first laboratory session in the same subjects rather than the EEG simultaneously
8 acquired during MRI data acquisition. The experimental protocol for both of these
9 sessions was identical with the exception of the acquisition of numerical ratings of pain
10 intensity of the laser stimuli.

11

12 EEG data were acquired using a 32 channel EEG cap (BrainCap MR, Easycap GmbH,
13 Germany) and MR compatible amplifier system (MRplus, BrainVision GmbH, Germany)
14 at 5KHz sampling rate using FCz as a reference electrode. An abrasive electrolyte
15 conducting gel was used between the electrodes and scalp to ensure electrode
16 impedances were kept below 5kΩ. Filtering (high-pass=0.5 Hz, low-pass filter=70 Hz,
17 notch=50Hz) was applied by the acquisition software (BrainVision Recorder, version
18 1.10), which also recorded the timings of the stimuli presentation and subjects' button
19 presses. Electrocardiographic signals (ECG) and vertical/horizontal eye movements
20 (VEOG/HEOG) were simultaneously recorded through an auxiliary device (BrainAmp ExG
21 MR, Brain Products GmbH, Germany) for offline removal of the blink artifacts.

22

23

24 **Statistics**

1

2 **Targeting loss of behavioral responsiveness**

3 Consistent loss of appropriate motor response to the cognitive word task was used to
4 define each individual's loss of behavioral response (LOBR) and allow the associated
5 changes in the neural processing of multi-sensory stimuli to be identified. A consistent
6 loss of motor response was defined as two consecutive missed responses to the
7 cognitive word task in both the FMRI and EEG analyses.

8

9 **Pre-processing and registration of MRI data**

10 FMRI-BOLD data were analyzed using FEAT (FMRIB's Expert Analysis Tool) version 6.0,
11 part of the FMRIB Software Library (FSL) version 6.0 (<http://www.fmrib.ox.ac.uk/fsl>)²².
12 Automated removal of non-brain tissue was performed using BET (Brain Extraction Tool,
13 FEAT v5.98), with further manual correction (if required) to remove any artefacts
14 introduced by the presence of the EEG electrodes. FMRI-BOLD data were truncated
15 around each individual's LOBR to include a total of 24 laser pain stimuli in the time-
16 window of interest; i.e. 12 stimuli prior to and 12 stimuli after each individual's first
17 consistent missed response to the word task (see Fig. 1B). Data were truncated to start
18 one whole brain acquisition (or volume) prior to the time of delivery of the first laser
19 stimulus in the time-window of interest and finish one volume before the onset of the
20 stimulus following the last (i.e. 24th) laser stimulus. This equated to an FMRI-BOLD data
21 run of approximately 12 minutes duration for each subject.

22

23 After truncation of the EPI data, data were pre-processed by performing motion
24 correction with MCFLIRT (Motion Correction FMRIB's Linear Registration Tool), spatial

1 smoothing using a Gaussian kernel of 5mm, global intensity normalization and applying
2 a high-pass temporal filter of 50s. The resulting functional scans were registered to each
3 individual's T1 high-resolution structural image using BBR (Boundary-Based
4 Registration)²³, and then to the Montreal Neurological Institute (MNI) standard brain
5 using FMRIB's Non-Linear Registration Tool (FNIRT). As the final stage of pre-processing,
6 MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent
7 Components) was used to identify cardiac, respiratory, movement and MR artefacts in
8 the FMRI-BOLD data. FMRIB's ICA-based Xnoiseifier (FIX) v1.06^{24,25} was used to
9 automatically classify these components and subsequently these non-signal
10 components were removed from the data.

12 **FMRI general linear modeling**

13 We performed an event-related FMRI analysis on a narrow time-window that was
14 centered on each individual's LOBR (see Fig. 1B). The denoised data was analyzed using
15 a two-level general linear model (GLM) to identify regions of brain activity associated
16 with the laser, tone and word task stimuli. Six regressors were included at the first level
17 to account for each stimulus type pre- and post-LOBR. Each of the stimuli were
18 modelled to be 1 second in duration and were convolved with a gamma haemodynamic
19 response function with mean lag = 6 ± 3 (SD) seconds. Further confound regressors
20 were used to account for the timing of each individual's motor responses and any
21 subject motion during the induction to loss of consciousness. Temporal filtering and
22 temporal derivatives were also applied to account for inter-subject and inter-area
23 differences in the haemodynamic response function.

1 This is an event-related design so the remaining unmodelled time serves as the baseline
2 period. As the first-level model depends on the timing of each individual's LOBR, each
3 model is different and there is some variability in number of events across volunteers.
4 The variation of the percentage signal change (and standard deviation) required in the
5 first-level contrasts was for the pre-LOBR > post-LOBR contrasts for the laser, word and
6 tone stimuli are $1.58\% \pm 0.14$, $2.52\% \pm 0.44$ and $2.04\% \pm 0.1$ respectively, as estimated
7 in FEAT ²⁶.

8
9 At second level, a mixed effects analysis was performed across subjects using FLAME
10 (FMRIB's Local Analysis of Mixed Effects) to identify the main effect of each regressor
11 and any differential processing of these stimuli across the LOBR transition. This higher-
12 level estimation method in FEAT estimates the higher-level parameter estimates and
13 mixed effects variance using a two-stage estimation technique that includes Metropolis-
14 Hastings Markov Chain Monte Carlo sampling. Cluster-based thresholding was used in
15 FEAT to reveal significant group-level brain activation that was corrected for multiple
16 comparisons across space. A Z statistic cluster threshold of 2.3 was used to define
17 contiguous clusters, then each cluster's estimated significance level (from Gaussian
18 Random Field theory) was compared with a cluster probability threshold of $p < 0.05$. For
19 visualization, the thresholded clusters in MNI space were applied to the very inflated
20 surface from the Human Connectome Project (HCP) Workbench tutorial dataset **Beta**
21 version 0.51 (www.humanconnectome.org)²⁷.

22 23 **FMRI conjunction analysis and timeseries plots**

1 A conjunction analysis was performed to identify any brain regions that demonstrated
2 significant reductions in stimulus-evoked activity across the loss of behavioral
3 responsiveness transition that were common for all stimulation types. The thresholded
4 Z-statistic maps for each of the differential laser, words and tones stimuli contrasts (i.e.
5 pre-LOBR>post-LOBR contrasts) were binarized and then multiplied together. This
6 created a mask in standard space that corresponded to where activity was commonly
7 reduced across the loss of behavioral responsiveness transition in response to all of the
8 multisensory stimuli. Again, for visualization, the brain regions identified by the
9 conjunction analysis in MNI space were resampled onto the very inflated surface from
10 the HCP Workbench tutorial dataset.

11
12 For demonstration purposes, the average stimulus-evoked percentage BOLD signal
13 change within the region identified by the conjunction analysis was calculated for each
14 type of stimulus pre- and post-LOBR. Firstly, the conjunction mask in standard space
15 was transferred back into each individual's subject space. For each individual, the
16 average stimulus-evoked BOLD timeseries within this mask were temporally re-sampled
17 using cubic spline interpolation then averaged across trials and subjects for each
18 stimulus type.

19 20 **Functional connectivity analysis**

21 A two-level GLM analysis was performed to identify changes in the functional
22 connectivity of the dAIC cluster (identified in the previous conjunction analysis) across
23 the LOBR transition. At the individual subject level, separate event-related GLMs were
24 set up for the time periods before and after the loss of behavioral response. These

models included regressors for the timing of 1) the delivery of all stimuli, 2) the motor responses where appropriate and 3) motion regressors. These first two regressors were used to account for the original task within the functional connectivity analysis. Consequently, they were convolved with a gamma haemodynamic response function with mean lag= 6 ± 3 (SD) seconds. Temporal filtering and temporal derivatives were applied as per the previous analysis described in the fMRI general linear modeling section. Six motion regressors were also included to account for any confounds of subject motion during the pre- or post-LOBR periods.

The final regressor included in the first-level analyses was the mean BOLD timeseries within the dAIC cluster for the time period of interest, i.e. either pre- or post-LOBR. This regressor was orthogonalised to the stimuli regressor and the response regressor where it was present. This regressor represents the main regressor of interest as it identifies any voxels within the brain that demonstrate altered functional connectivity with the dAIC that are not already explained by activation due to the stimuli or responses.

At second level, a mixed effects paired-t test analysis was performed across subjects using FLAME to identify any changes in the dAIC's functional connectivity around the LOBR transition. The individual dAIC whole-brain connectivity contrasts for pre- and post-LOBR periods for the $n = 15$ subjects were used as input to the model, resulting in 30 inputs in total at second level. The higher-level GLM was set-up with one regressor to identify the change in dAIC's functional connectivity across the LOBR transition, and 15 further regressors to control for each subject's mean effect. Cluster-based

1 thresholding ($Z=2.3$, $p<0.05$) and multiple comparisons correction across space was
2 used, as described in the fMRI general linear modeling section.

3 4 **Frontoparietal EEG synchrony analysis**

5 In order to corroborate our fMRI findings, we examined changes in frontoparietal EEG
6 synchrony at electrode locations corresponding to the brain regions that had been
7 identified to have reduced functional connectivity with the dAIC around LOBR. As the
8 BOLD signal typically detects changes in activity over time courses of perhaps 3-15s, we
9 sought to use EEG methods that varied over a comparably slow time course. With the
10 proviso of some distortion by the filtering effects of the skull, scalp, and electrode
11 interface; the peaks of the slow EEG oscillations tend to reflect cortical pyramidal cell
12 depolarisation and high neuronal activity whereas the EEG troughs indicate
13 hyperpolarisation and relative inactivity.

14
15 The main problem with direct current recording methods is that of low frequency
16 electrode drift. However the slow modulation of EEG activity can be detected indirectly
17 by examining the amplitude envelope of fluctuations in the power of the traditional EEG
18 waves²⁸. The modulation of the envelopes of the lower frequencies has been shown to
19 be a reasonable predictor of fMRI connectomes²⁹, and thus might reasonably be
20 considered to be an index of fluctuations in cortical activity, somewhat analogous to
21 those seen in the BOLD signal. We used the EEG data that was recorded during an
22 identical propofol administration protocol outside the scanner in the same subjects
23 (bench EEG data). The bench EEG has significantly lower noise levels than the scanner
24 EEG data as is required to detect subtle changes in long range correlations. Although

1 most published methods use a bandpass-filtered Hilbert envelope method, we found
2 that a short-time Fourier transform was a simple method of quantifying the slow
3 fluctuations in power over about 3-15s.

4
5 In order to spatially localise the EEG signal, we used a nearest neighbor modified Hjorth
6 Laplacian derivation. This involved subtracting the mean values of the surrounding
7 three electrodes from the electrode that overlay the regions of interest. We then
8 compared the changes in synchrony between the electrode lying over the right
9 dorsolateral prefrontal cortex (Fp2) and the right inferior parietal regions (P8). As a
10 control site, we also examined the synchrony between electrodes overlying regions of
11 the cortex that were close to, but distinct from, the regions of interest; namely by
12 comparing the synchrony of the dorsolateral prefrontal cortex (Fp2) and the midline
13 parietal cortex (Pz).

14
15 Data were exported from BrainVision Analyser Version 2.0 (BrainProducts GmbH,
16 Munich, Germany) and analyzed in Matlab (Mathworks Inc., USA). EEG data were
17 downsampled to 125HZ and truncated to examine a period of 6 minutes before until 6
18 minutes after the time of LOBR for each subject. For each of the EEG channels, we used
19 the short-term Fourier transform 'spectrogram.m' function (window=4sec,
20 overlap=3sec, 0.25Hz resolution) to obtain the mean EEG power (expressed in decibels)
21 between 0.5 to 45Hz. The synchrony between the two regions was quantified by using a
22 simple zero-lag Pearson's cross correlation coefficient (r) applied to 60 sec sections of
23 slow wave power (0.5 to 1.5Hz). Statistical comparisons were done using paired t-tests

- 1 on the mean values for the 6 minutes before the LOBR with the mean of the 6 minutes
- 2 after LOBR.

Results

Demographics

Sixteen subjects participated in the bench EEG and the EEG-FMRI session. For one subject, the induction to loss of consciousness was stopped as the participant developed an obstructive respiratory pattern during the EEG-FMRI session. For another subject, FMRI data acquisition was interrupted as the participant was removed from the scanner and repositioned once the safety of the individual was re-established. Data from the first FMRI acquisition was sufficient to perform this analysis as the subject had lost responsiveness several minutes earlier. Consequently FMRI and bench EEG data are presented for the same 15 healthy volunteers (8 female, aged 19-43 years, mean age \pm standard deviation of 28.7 ± 7.3 years).

Loss of behavioral responsiveness (LOBR)

The loss of an appropriate motor response to an auditory word discrimination task was used to define LOBR. In the scanner, the loss of behavioral responsiveness was abrupt in 14 of the 15 volunteers and the mean estimated propofol effect site concentration (ESC) at which it occurred was $1.27 \mu\text{g/ml}$ with 95% confidence limits (CL) of 0.92 - $1.62 \mu\text{g/ml}$. The mean propofol ESC \pm SD increase for all subjects across the narrow LOBR time-window was $1.1 \pm 0.09 \mu\text{g/ml}$ with a range of 1.0 - 1.2 .

Similarly for the bench EEG data, the mean ESC \pm SD at which loss of behavioral responsiveness occurred was $1.53 \pm 0.6 \mu\text{g/ml}$ with 95% confidence limits (CL) of 1.20 -

1 1.86µg/ml. This was equivalent to a propofol ESC increase for all subjects across the
2 12minute window used for the EEG analysis of 1.2µg/ml.

3

4 **Reduction in brain activity associated with LOBR under propofol anesthesia**

5 The fMRI analysis on a narrow time-window (of approximately 12 minutes in total) that
6 was centered on each individual's LOBR allowed us to identify specific changes in the
7 processing of auditory tones, words, and noxious pain stimuli associated with this
8 transition. This window length was used to provide a compromise between the number
9 of events required for adequate signal to noise in the fMRI findings but also to limit the
10 temporal window of investigation so that we can still observe the distinct state change
11 that occurs at LOBR. By examining stimulus-evoked fMRI blood-oxygen level dependent
12 (BOLD) changes, we demonstrate that propofol anesthesia did not disrupt the
13 stereotypical perceptual activation patterns around the LOBR transition (Fig. 2). For
14 example, by examining the mean effects before and after LOBR separately in response
15 to laser stimulation, we found that widespread thalamocortical processing persisted
16 after LOBR. Activity was observed after LOBR in response to laser stimulation in brain
17 regions known to be responsible for conscious pain perception, including the thalamus
18 and the insula, anterior cingulate and somatosensory cortices. Similarly, thalamocortical
19 processing persisted past LOBR for both types of auditory stimuli with sensory-specific
20 perceptual activation patterns seen for word and (to a lesser degree) for tone stimuli.

21

22 In addition, a comparison of the mean stimulus-evoked activity before and after each
23 individual's loss of behavioral response (i.e. contrasting pre-LOBR and post-LOBR
24 periods), showed significant localized reductions across the transition (Fig. 3, Table 1). In

contrast, no brain regions were found to be more active to any stimuli after LOBR than before (i.e. the post-LOBR > pre-LOBR contrasts).

By performing a conjunction of these contrasts across all sensory modalities, we demonstrated that propofol suppressed activation in a specific region of the right dAIC after LOBR (Fig. 4A). For demonstration purposes, the average time-locked BOLD percentage signal change for each stimulus type within this dAIC region is presented for the time-periods before and after LOBR (Fig. 4B) to confirm the suppression of stimulus-evoked activity.

Functional connectivity changes of the dorsal anterior insula cortex

A functional connectivity analysis was performed to identify changes in functional connectivity of the dAIC cluster across the LOBR transition. Reductions in the functional connectivity of the dAIC were observed in the dorsolateral prefrontal cortex (dlPFC), inferior parietal lobule (IPL) and cerebellum after loss of responsiveness (Fig. 5, Table 2). No brain regions were found to increase their functional connectivity with the dAIC after the LOBR transition.

Frontoparietal EEG synchrony changes around LOBR

We found that the synchrony between the electrode pair that most closely overlaid the dorsolateral prefrontal cortex and the inferior parietal lobule (Fp2 and P8) decreased significantly around the point of LOBR as predicted by the functional connectivity results ($p=0.007$, t-test) (Fig. 6). The synchrony between the Fp2 and Pz electrodes, which are

- 1 separated by a similar distance, but correspond to the nearby regions of the midline
- 2 parietal cortex, did not show a significant decrease ($p=0.26$, t-test).
- 3
- 4
- 5

Discussion

In this paper, we have focused on the changes in neural activity associated with loss of behavioral responsiveness. Our results suggest that the dorsal anterior insula cortex (dAIC) could be a key cortical region that underpins loss of behavioral responsiveness (LOBR) under anesthesia. We found that activity within this brain region was commonly reduced in response to both painful and two types of auditory stimuli around the point of LOBR in healthy volunteers (Fig. 3). Identification of anterior insula suppression at LOBR is perhaps unsurprising given that the insula is the most frequently reported area of activation across all neuroimaging studies³⁰. Furthermore, there is a myriad of work postulating the dorsal anterior insula division specifically to be the embodiment of the 'sentient self'³¹ and a critical hub of a salience/threat detection network along with the anterior cingulate cortex (ACC)³².

Recent meta-analyses^{10,33} have also suggested the region of the dAIC identified in our conjunction analysis to be in the cognitive rather than sensorimotor domain, supporting the idea that suppression of dAIC activity is related to a loss of volition/willingness rather than the ability to produce motor responses. We should note that subjects in the study were instructed to respond only after the auditory word stimuli were presented; therefore, any loss of responsiveness does not necessarily indicate an inability to move. Indeed, examination of the motion correction data indicates that many subjects continued to move slightly after they had lost their volitional response. We hypothesized that suppression of activity in a cortical gate around LOBR (such as the dAIC) could act as the seed for the subsequent breakdown of frontoparietal feedback and the loss of information integration seen at deeper levels of anesthesia.

1 Subsequently, we have shown using FMRI that dAIC's functional connectivity to
2 frontoparietal regions is reduced after LOBR (Fig. 5). Specifically, we found that the
3 dorsolateral prefrontal cortex (dLPFC) and inferior parietal lobule (IPL) had reduced
4 functional connectivity with the dAIC. Interestingly, we also observed a reduction in
5 dAIC's functional connectivity with the cerebellum around LOBR. The center of gravity
6 for this cluster was located in the Crus I region, which is known to be involved in
7 executive functions through its functional connectivity with prefrontal regions of the
8 brain^{34,35}.

10 The dLPFC, identified in the functional connectivity analysis, forms part of the executive
11 control network and is highly implicated in working memory and decision-making. The
12 IPL cluster we identified covers the PF, Pft and PM divisions defined by Casper and
13 colleagues³⁶, with its center of gravity within the PM division. These regions of the IPL
14 have been shown to have the strongest interactions with dLPFC through a combination
15 of diffusion tractography-based parcellation and human resting-state data analyses³⁷.
16 Interestingly, the PM region of the IPL has also been shown to be more active when
17 subjects switch their response strategy³⁸. This fits well in the context of our
18 experimental data where the subjects' loss of responsiveness was defined according to
19 a cognitive auditory word task where subjects were being asked to choose between two
20 options (i.e. when SAME or DIFFERENT pairs of words were presented).

22 Our finding that dAIC suppression at LOBR is associated with altered stimulus-evoked
23 functional connectivity in frontoparietal regions fits well with previous findings and
24 global theories of consciousness that postulate that loss of consciousness (or often

more correctly loss of responsiveness) is based on the disruption of frontoparietal
 communication^{3,6,12,39–41}. Further support for this being (either directly or indirectly)
 mediated by suppression of the dAIC is added through our EEG power synchrony
 analysis (see Fig. 6). Whilst, EEG techniques have poor spatial resolution compared to
 that of FMRI, these results nonetheless suggest that loss of long range synchrony
 around the point of LOBR is greatest between these relatively localized regions
 identified by the functional connectivity analysis (i.e. the IPL and dLPFC).

Due to anatomy, it is difficult to identify any localized changes in dAIC activity around
 LOBR using scalp EEG without using source localization. However, the fact that these
 EEG data reproduce our dLPFC and IPL findings gives us additional confidence in the
 widespread applicability of our results, especially as these data were collected in the
 same subjects but in a different recording session. We also note that these effects could
 be seen using a medium density scalp montage and did not require any source
 localization. It is also important to note the shape of the curves in Figure 6 is suggestive
 of an abrupt decrease in synchrony around LOBR. However, because we were
 examining correlations between power fluctuations in the slow wave frequency band
 (0.5-1.5Hz), the time resolution is limited to around 60 seconds and limits our ability to
 assess the true shape of this curve. This resolution is comparable to that with which we
 are able to assess LOBR, due to the variability in timing of the presentation of the
 auditory word task.

It has been postulated that the disruption of synchronous activity is indicative of
 disruption in the interaction between regions, and hence linked to a disruption in

functional inter-regional communication⁴². Whilst, our methods do not have the temporal resolution to reliably infer directionality or causality, we would suggest that the observed loss of synchrony between these frontoparietal regions potentially indicates failed information transfer between the regions caused by the increasing concentrations of propofol.

Selfhood may be defined as the experience of being a distinct entity that is capable of self-control and attention⁴³. On the basis of the results presented and the proposed role of the dorsal anterior insula division as the site of the sentient self, we hypothesize that general anesthesia challenges an individual's selfhood through suppression of the dAIC. We propose that this LOBR transition lies at the boundary of the transitive and intransitive definitions of consciousness⁴⁴, i.e. where individuals may *be conscious* but are not necessarily *consciously aware* that stimuli relate to them; namely, when an individual loses selfhood. A central component of selfhood is the experience of body ownership, which is thought to arise from predictive (top-down) integration of (bottom-up) multisensory information from interoceptive and exteroceptive domains^{43,45}. When this is lost, as potentially occurs at LOBR through direct (or indirect) suppression of dAIC activity, there is no need to respond to incoming sensory events because they are not perceived as happening 'to me'.

In clinical practice, patients typically lose behavioral responsiveness to external auditory stimuli at concentrations of general anesthetic around 30% of those required for full general anesthesia (as measured by lack of somatic movement to surgical incision). Over the years there has been indirect evidence of substantial perceptual processing in

1 lightly anesthetized patients; namely a high incidence of dreaming⁴⁶, implicit memory
2 formation⁴⁷, and even explicit recall of intraoperative events⁴⁸. Patients, and also
3 experimental study participants⁴⁹, often report a loss of engaged consciousness or
4 disconnection from the environment. Importantly, our dAIC finding is supported by a
5 recent case study in an awake epilepsy patient that found electrical (rather than
6 pharmacological) disruption of the dAIC/claustum region led to a reproducibly
7 disrupted consciousness and loss of behavioral responsiveness⁵⁰. In reference to
8 disorders of consciousness patients, the ability to engage with the outside world is
9 ultimately used to discriminate patients as minimally conscious rather than in a
10 persistent vegetative state. Our findings linking the loss of behavioral responsiveness
11 associated with suppression of activity within the dorsal anterior insula potentially
12 provides the link between the internal and external awareness networks that have been
13 discussed in the context of these patients⁵¹.

14
15 Interestingly, we did not observe any change in the activity of the ACC around the LOBR
16 transition (see Figures 2 and 3, Table 1), despite its proposed role alongside the anterior
17 insula as part of the salience detection network³². This may be due to limited statistical
18 power, either due to the experimental design and/or the relatively small study sample
19 size. The signal to noise ratio (SNR) in stimulus-evoked fMRI experiments depends upon
20 many factors, for example: the number, duration, and intensity of stimulation, the data
21 acquisition parameters, and the analysis methods used. Given the need to remain
22 temporally close to the LOBR transition of interest, the number of word stimuli was
23 potentially close to minimum in our experimental design. The final sample size for our
24 analysis was equivalent to⁶ or in excess of other contemporaneous fMRI experiments

1 performed under anesthesia^{9,17-19}, which in some way compensates for the reduced
2 SNR at an individual level. However, a secondary study with optimal statistical efficiency
3 is required to identify whether other brain regions exist, in addition to the dAIC, which
4 have functional relevance for LOBR but with smaller effect sizes. Further work will also
5 be required to assess if our current dAIC finding holds for other anesthetic agents in
6 addition to propofol.

7
8 In conclusion, our results show for the first time the explicit neurobiology that underlies
9 loss of behavioral responsiveness under general anesthesia. We conclude that
10 pharmacological lesioning of the right dAIC by propofol anesthesia is (either directly or
11 indirectly) responsible for the measurable loss of volitional behavioral response seen in
12 clinical practice. We have provided evidence that this suppression of dAIC activity
13 results in reductions of functional connectivity and EEG synchrony in frontoparietal
14 brain regions around LOBR. In summary, the dAIC provides a potential cortical gate for
15 loss of behavioral responsiveness observed under general anesthesia.

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TABLES

Table 1: Cluster location for brain regions demonstrating increased activity prior to loss of behavioral responsiveness in response to multisensory stimuli

The maximum Z-statistic and the location of the center of the gravity (Z-COG) for each cluster are presented for each differential contrast around loss of behavioral response (i.e. pre-LOBR > post-LOBR contrasts) for the pain, words and tone stimuli. Mixed-effects group analysis (n=15) with cluster corrected threshold of Z=2.3 and significance level of $p < 0.05$. Abbreviations: Z-max – maximum Z-statistic, L - left, R – right, dAIC – dorsal anterior insula cortex, STG = superior temporal gyrus and PCC – posterior cingulate cortex. Coordinates are expressed in millimeters (mm) in Montreal Neurological Institute (MNI) 152 standard space.

Contrast	Cluster no.	Size (voxels)	Cluster location	Side	Z-max	Z-COG coordinates (mm)			Sign.
						x	y	z	
Pain	1	421	dAIC	R	3.33	38.2	14.2	3.94	0.0016
Words	1	694	STG/Wernicke's	R	3.27	52.3	-25.3	9.49	<0.001
	2	369	Heschl's gyrus	L	3.43	-45.8	-24.8	10.5	<0.001
	3	244	dAIC	R	3.05	32.8	23.2	-2.11	0.013
Tones	1	1110	PCC	R	3.28	2.13	-38.2	36.4	<0.001
	2	577	Precuneus	R	3.59	9.78	-66.9	41.1	<0.001
	3	512	dAIC	R	3.66	32.2	21.8	-0.55	<0.001
	4	469	dAIC	L	3.84	-33.2	17.6	1.91	0.0013

Table 2: Cluster location for brain regions demonstrating increased functional connectivity with the dorsal anterior insula cortex (dAIC) cluster prior to loss of behavioral responsiveness

The maximum Z-statistic and the location of the center of the gravity (Z-COG) for each cluster are presented for the change in the dAIC's functional connectivity around loss of behavioral response (i.e. pre-LOBR > post-LOBR contrasts). Mixed-effects group analysis (n=15) with cluster corrected threshold of Z=2.3 and significance level of $p < 0.05$.

Abbreviations: Z-max – maximum Z-statistic, L - left, R – right, PFC – prefrontal cortex, VI – Cerebellar Lobule VI. Coordinates are expressed in millimeters (mm) in Montreal Neurological Institute (MNI) 152 standard space.

Cluster location	Size (voxels)	Side	Z-max	Z-COG coordinates (mm)			Sign.
				x	y	z	
dorsolateral PFC	482	R	3.09	33.6	54.6	25.4	<0.001
cerebellum (Crus I, VI)	349	R	3.23	27.8	-72.4	-23.5	0.004
inferior parietal lobule	237	R	3.25	53.4	-41.1	40.8	0.04

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1 _____ **APPENDICES**

2 2 None

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4 _____ **COPIES OF ANY LISTED IN-PRESS PAPERS**

5 5 None

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Figure 1: Targeting loss of behavioral response (LOBR) under anesthesia

A. Ultraslow induction to and recovery from propofol-induced loss of consciousness.

Laser (red), tone (green), and word discrimination task (blue) stimuli were delivered during a target-controlled infusion to maximum effect site concentration (ESC) of

4 μ g/ml. B. Targeted functional magnetic resonance imaging analysis to identify brain

regions with altered activity associated with LOBR (black dashed line). LOBR was

defined as consistent loss of motor response (purple circles) to the auditory word

task, where individuals responded if the two words presented were the same or

different. Each individual's functional magnetic resonance imaging (fMRI) data was

truncated (grey box) to include 12 laser stimuli pre- and 12 post-LOBR.

Figure 2: Brain regions demonstrating activity in response to multi-sensory stimuli before and after loss of behavioral responsiveness (LOBR)

A. Each panel shows sagittal, coronal and axial brain slices demonstrating the stimulus-evoked blood oxygen level dependent (BOLD) activity for n=15 subjects in response to laser pain (red), word (blue) and tone (green) stimuli pre- and post-LOBR. B. BOLD activity elicited by the motor responses (purple) to the cognitive word task for n=15 subjects. Mixed effects analysis: cluster corrected thresholded $Z=2.3$ and $p < 0.05$.

Figure 3: Brain regions demonstrating reduced stimulus-evoked activity after LOBR

The panel shows increased fMRI-BOLD activity before compared with after loss of responsiveness (LOBR) in response to A) laser pain (red), B) words (blue) and C) tones (green) stimuli in 15 healthy subjects (mixed effects, cluster corrected threshold $Z=2.3$, $p<0.05$). In contrast, no brain regions were found to be more active to any stimuli after LOBR than before (i.e. post-LOBR > pre-LOBR contrasts).

Figure 4: Dorsal anterior insula cortex (dAIC) activity is lost to multisensory stimuli after LOBR

A: Conjunction of the group (n=15) differential stimulus-evoked responses around loss of behavioral responsiveness (LOBR) demonstrates reduced activity of right dAIC (yellow) region to all multisensory stimuli. B: Average stimulus-locked blood oxygen level dependent (BOLD) percentage signal changes across individuals (n=15) within the dAIC region for each stimulation type for pre-LOBR (solid line) and post-LOBR (dashed line) time-periods and the associated standard errors.

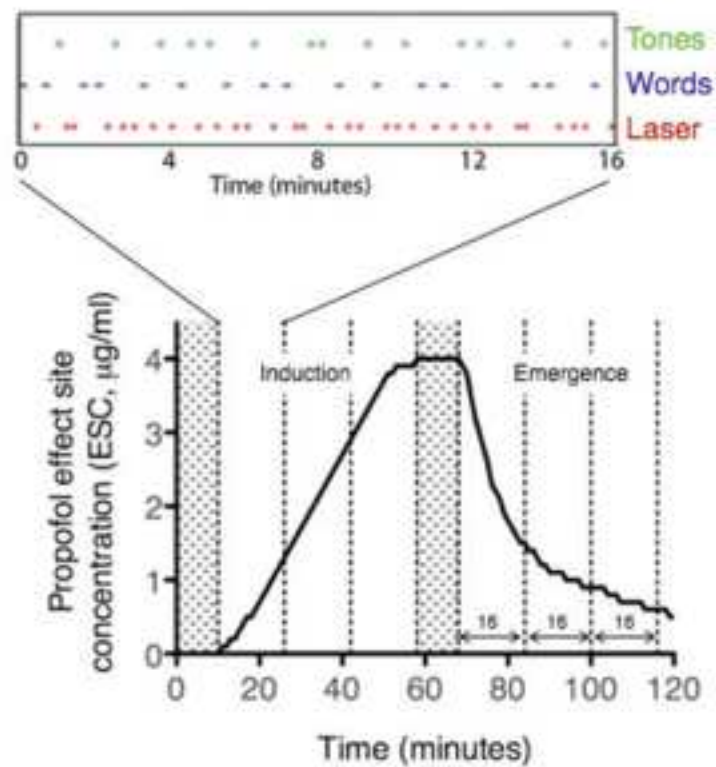
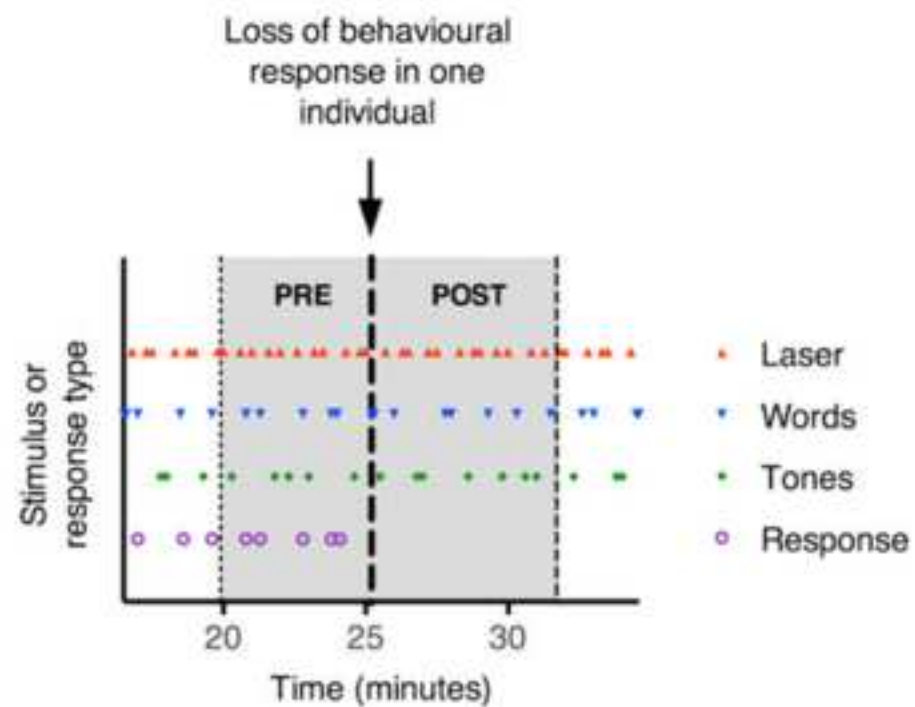
Figure 5: Changes in the functional connectivity of the dorsal anterior insula cortex (dAIC) region around loss of behavioral responsiveness

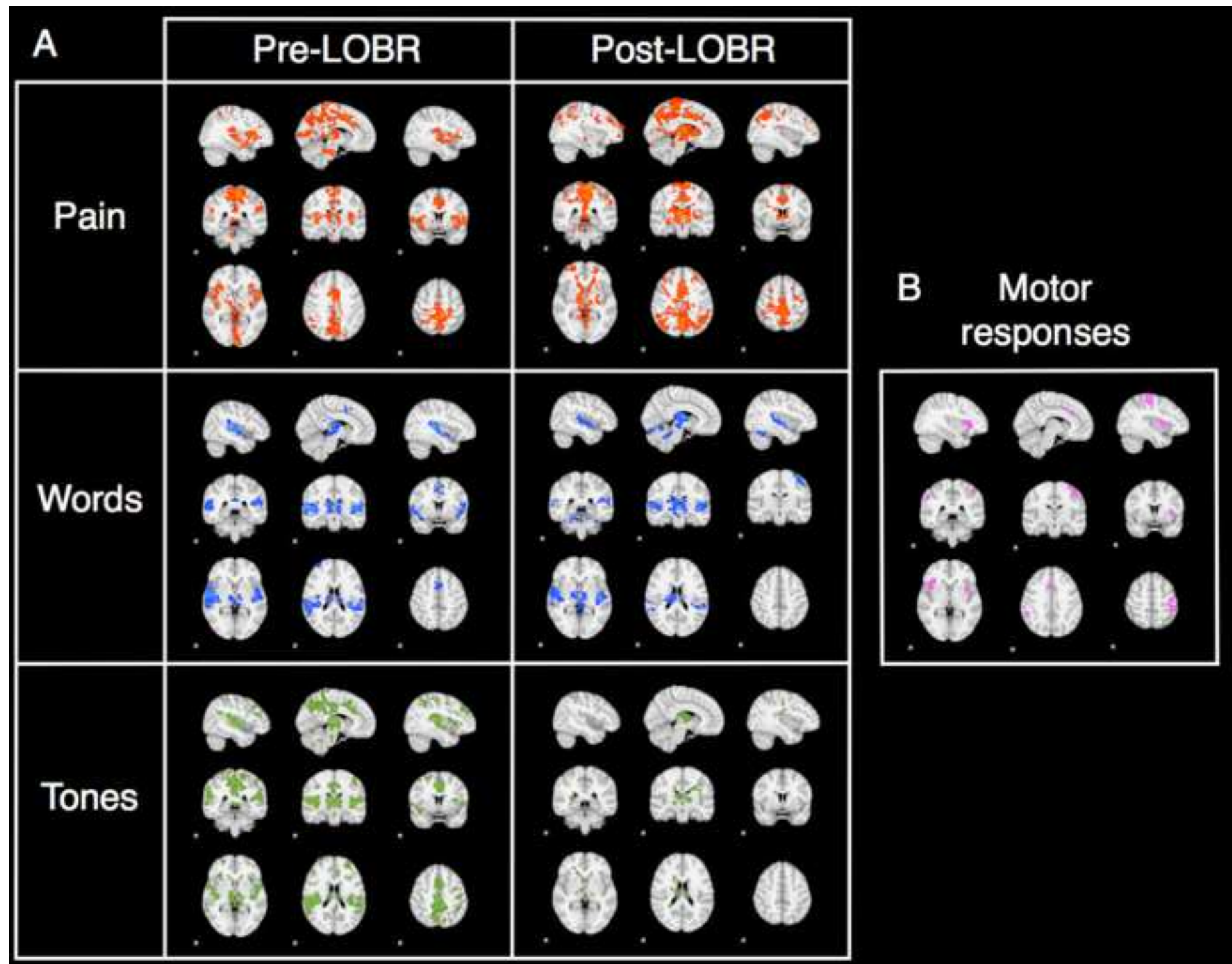
A mixed effects whole-brain analysis in n=15 volunteers revealed localised changes in functional connectivity of the dorsal anterior insula cortex (dAIC) region in the right inferior parietal lobule (IPL - left panel), right dorsolateral prefrontal cortex (dLPFC - middle) and right Crus I/Lobule VI regions of the cerebellum (right). The corresponding z-axis coordinates are expressed in millimeters (mm) in Montreal

Neurological Institute (MNI) 152 standard space. The results presented are cluster corrected thresholded at $Z=2.3$ and significance level of $p<0.05$.

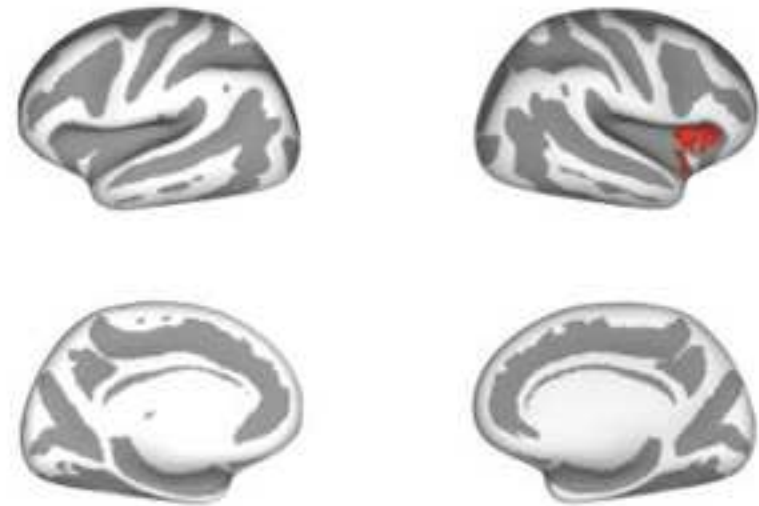
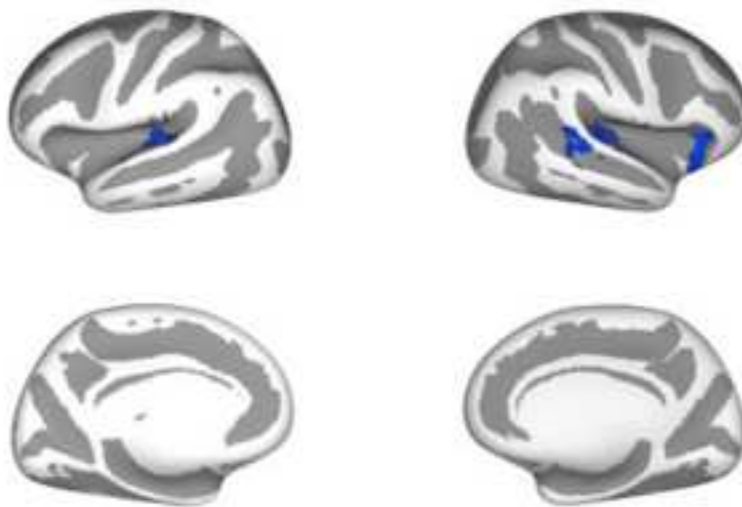
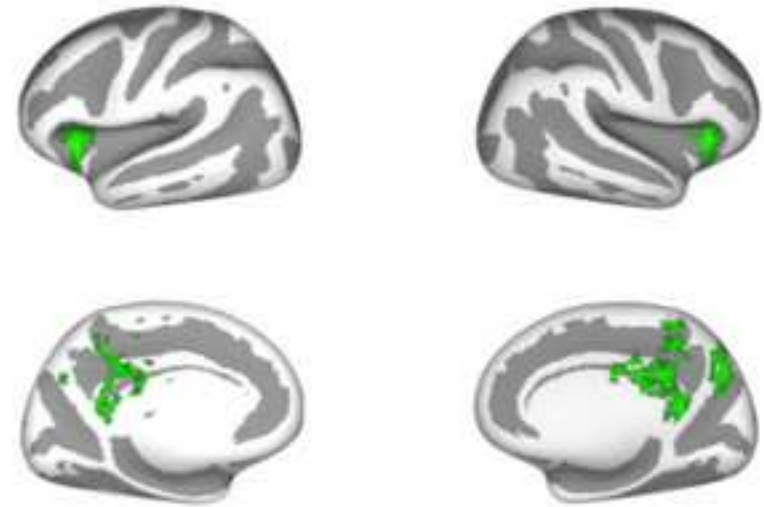
Figure 6: Reductions in frontoparietal EEG power synchrony around loss of behavioral responsiveness

Electroencephalographic (EEG) power synchrony between Fp2-P8 (in black) and Fp2-Pz (grey) channel pairs are presented as mean \pm standard error for n=15 subjects during the laboratory EEG study for the time period 6 minutes before and 6 minutes after loss of behavioral responsiveness. Loss of behavioral response (LOBR) is at shown at time = 0 so that the grey region corresponds to the time period post-LOBR. Correlation is expressed as the Pearson's correlation coefficient (r) of the slow wave power (0.5-1.5Hz) fluctuations.

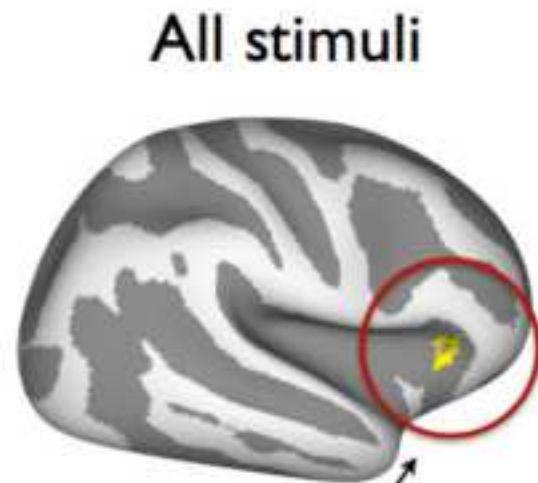
A**B**



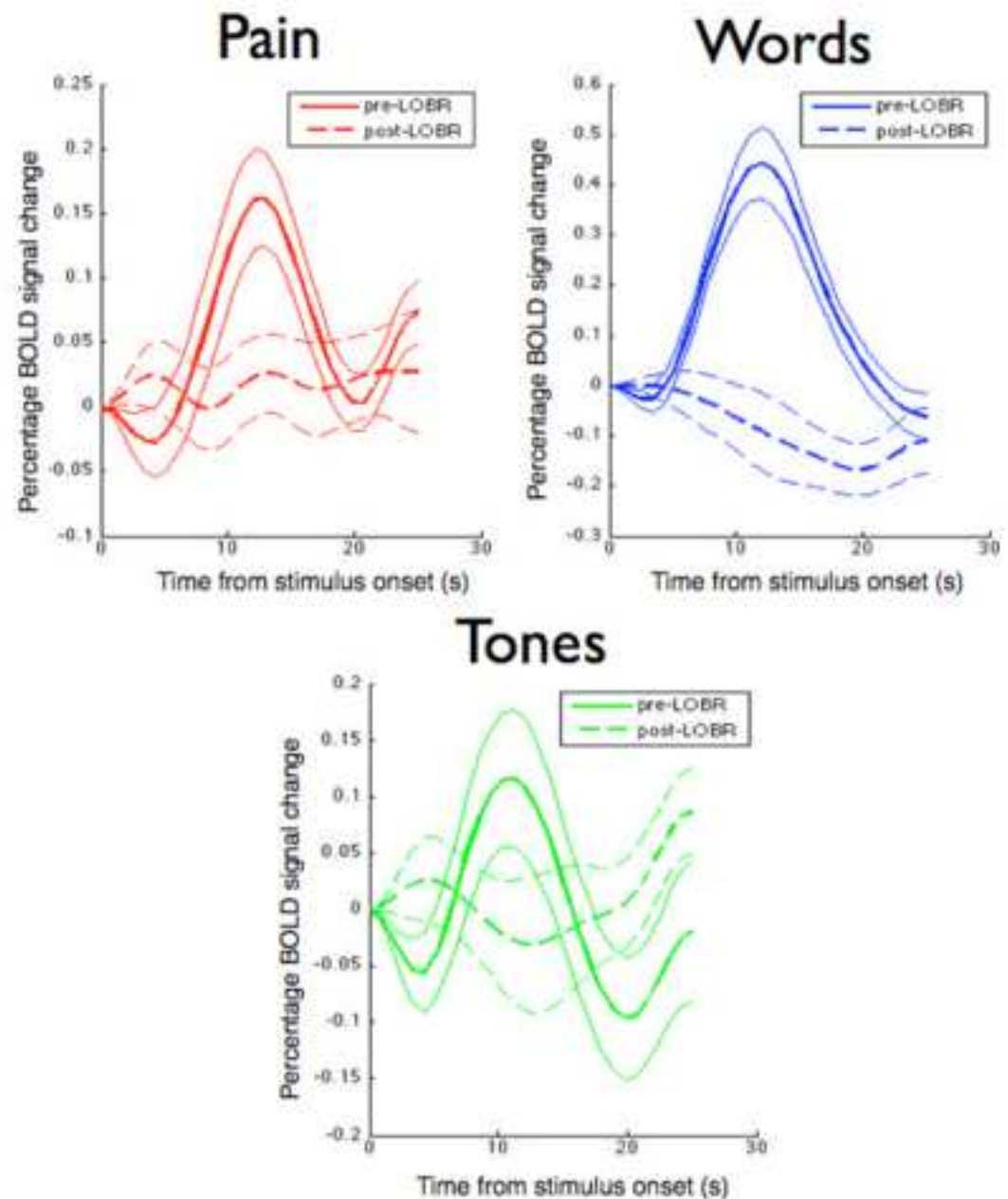
Stimulus-evoked
brain activity
pre-LOBR > post-LOBR

A**Pain****B****Words****C****Tones**

A Conjunction analysis pre-LOBR > post-LOBR



B Percentage BOLD signal change in dAI region



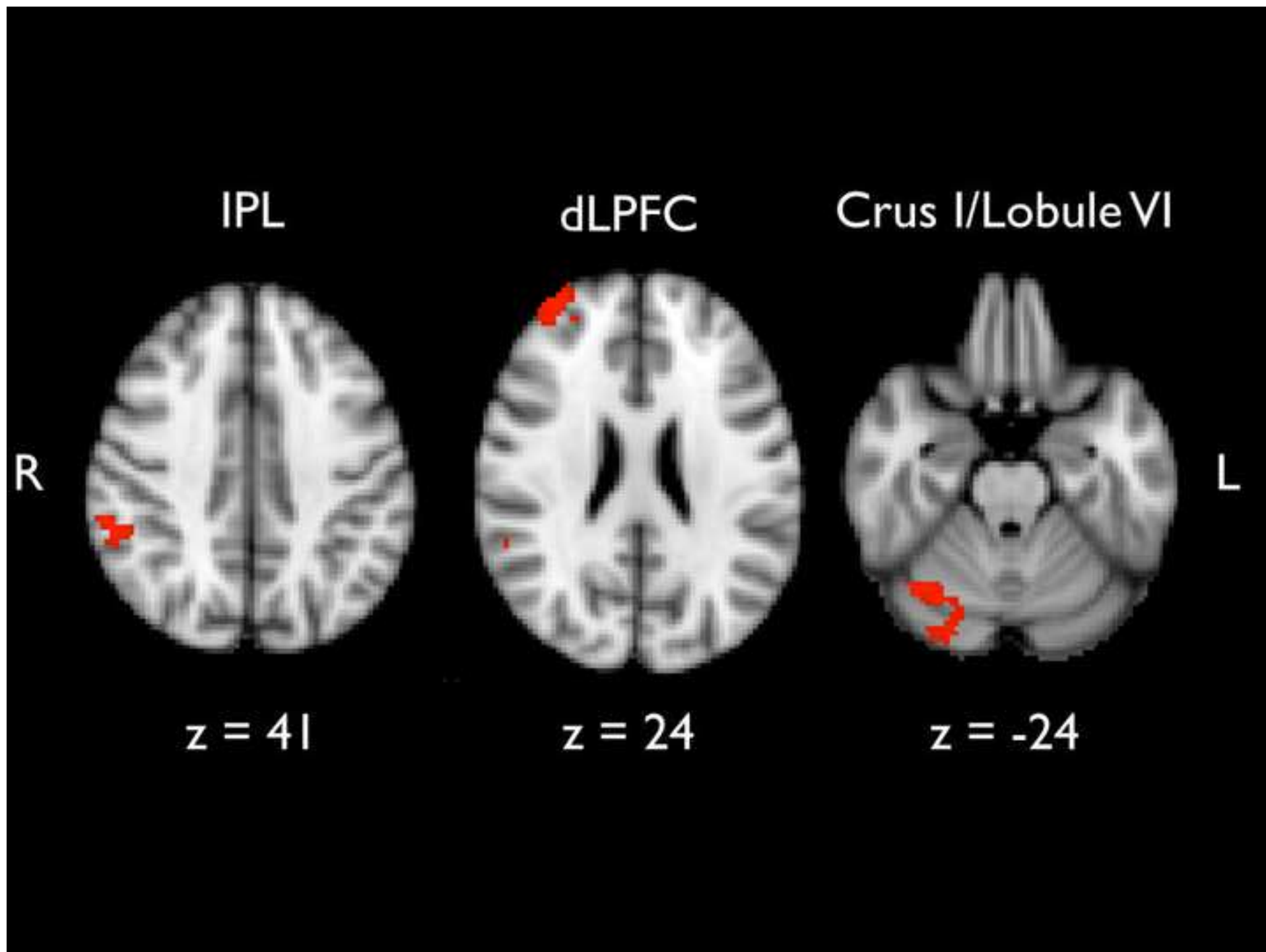


Figure 6

