doi:10.1093/brain/awy070 BRAIN 2018: 141; 1404–1421 | 1404

BRAIN
A JOURNAL OF NEUROLOGY

Characterization of EEG signals revealing covert cognition in the injured brain

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See Boly and Laureys (doi:10.1093/brain/awy080) for a scientific commentary on this article.

Patients with severe brain injury are difficult to assess and frequently subject to misdiagnosis. 'Cognitive motor dissociation' is a term used to describe a subset of such patients with preserved cognition as detected with neuroimaging methods but not evident in behavioural assessments. Unlike the locked-in state, cognitive motor dissociation after severe brain injury is prominently marked by concomitant injuries across the cerebrum in addition to limited or no motoric function. In the present study, we sought to characterize the EEG signals used as indicators of cognition in patients with disorders of consciousness and examine their reliability for potential future use to re-establish communication. We compared EEG-based assessments to the results of using similar methods with functional MRI. Using power spectral density analysis to detect EEG evidence of task performance (Two Group Test, $P \leqslant 0.05$, with false discovery rate correction), we found evidence of the capacity to follow commands in 21 of 28 patients with severe brain injury and all 15 healthy individuals studied. We found substantial variability in the temporal and spatial characteristics of significant EEG signals among the patients in contrast to only modest variation in these domains across healthy controls; the majority of healthy controls showed suppression of either 8–12 Hz 'alpha' or 13–40 Hz 'beta' power during task performance, or both. Nine of the 21 patients with EEG evidence of command-following also demonstrated functional MRI evidence of command-following. Nine of the patients with command-following capacity demonstrated by EEG showed no behavioural evidence of a communication channel as detected by a standardized behavioural assessment, the Coma Recovery Scale – Revised. We further examined the potential contributions of fluctuations in arousal that appeared to co-vary with some patients' ability to reliably generate EEG signals in response to command. Five of nine patients with statistically indeterminate responses to one task tested showed a positive response after accounting for variations in overall background state (as visualized in the qualitative shape of the power spectrum) and grouping of trial runs with similar background state characteristics. Our findings reveal signal variations of EEG responses in patients with severe brain injuries and provide insight into the underlying physiology of cognitive motor dissociation. These results can help guide future efforts aimed at re-establishment of communication in such patients who will need customization for brain–computer interfaces.

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Received July 19, 2017. Revised January 19, 2018. Accepted January 23, 2018. Advance Access publication March 19, 2018

Keywords: arousal; brain–computer interface (BCI); consciousness; electroencephalography (EEG); traumatic brain injury (TBI)

Abbreviations: BCI = brain–computer interface; BOLD = blood oxygenation level-dependent; CMD = cognitive motor dissociation; CRS-R = Coma Recovery Scale – Revised; DOC = disorder of consciousness; TGT = Two Group Test

Introduction

In past decades, it has become recognized that high level, covert cognition may be present in patients whose bedside evaluation appears consistent with the vegetative state or minimally conscious state (Owen *et al.*, 2006; Monti *et al.*, 2010; Bardin *et al.*, 2011; Goldfine *et al.*, 2011). Identification of covert cognition in such disorder of consciousness (DOC) patients is important, as it can lead to improved rehabilitative efforts that may foster recovery or even, in some instances, avert premature withdrawal of life-sustaining therapies (Fins *et al.*, 2007; Giacino *et al.*, 2014; Fins, 2015). In this context, perhaps the most urgent and concerning problem is that of persons with a latent capacity to restore a communication channel who continue to go unidentified (Fins and Schiff, 2016; Thengone *et al.*, 2016). An operational definition of 'cognitive motor dissociation' (CMD) has been developed for patients who demonstrate sharp dissociation of an inability or extremely limited ability to move but preservation of higher-level cognition in the form of reliable command-following, as detected with functional MRI, EEG or other non-invasive measures (Schiff, 2015).

Identification of CMD should instigate an immediate effort to restore communicative abilities, either through a brain–computer interface (BCI) or behavioural means, if residual motor capacities are identified (Fins, 2015; Schiff, 2015; Fins and Schiff, 2016). However, testing the severely brain-injured population for covert cognitive capacities is difficult and subject to many constraints. Standardized behavioural exams have been shown to be susceptible to significant inaccuracy in the diagnosis of DOCs (Schnakers *et al.*, 2009; Wannez *et al.*, 2017). Moreover, obtaining usable functional MRI or EEG signals from DOC patients is often severely limited by movement and muscle artefacts (Gill-Thwaites, 2006; Bardin *et al.*, 2011; Laureys and Schiff, 2012; Goldfine *et al.*, 2013). Fluctuations in overall arousal state can also markedly alter the behavioural capacity of DOC patients (Schiff *et al.*, 2007; Williams *et al.*, 2013) and may influence accuracy of both behavioural and non-invasive measurements with functional MRI or EEG (Casali *et al.*, 2013; Gibson *et al.*, 2014; Wannez *et al.*, 2017).

Furthermore, ultimate restoration of communication in CMD patients is uncertain (Thengone *et al.*, 2016) and, unlike patients in locked-in state who have isolated interruption of motor outflow pathways, CMD is characterized by concomitant injuries across the cerebrum that may significantly limit ability to harness a potential communication channel (Schiff, 2015; Fins and Schiff, 2016). Additionally, locked-in state patients demonstrate new challenges of sensitivity to arousal regulation and limitations of maintaining two-way communication once loss of overt motor function leads to the complete locked-in state (Chaudhary *et al.*, 2017).

Recent studies suggest that preservation of covert cognition in the form of a capacity to generate mental imagery on command co-exists with relatively well-preserved corticothalamic physiological activity that often remains unmeasured in a large portion of severely injured individuals (Forgacs *et al.*, 2014; Stender *et al.*, 2014, 2016). Thus, better characterization of the cortical activity indicative of covert cognition in this population can meaningfully guide meeting the future challenges of moving from non-invasive measurement of a potential binary switch utilizable as a communication channel to establishing readiness for a BCI or effective two-way communication (Thengone *et al.*, 2016).

In the present study, we sought to characterize the brain signals that established methods have taken as evidence of covert cognition in patients with severe brain injuries. We use both functional MRI and quantitative EEG methods that have been shown to reliably detect command-following capacity in DOC patients (Bardin *et al.*, 2011; Goldfine *et al.*, 2011). We compare the response characteristics of 15 healthy controls performing quantitative EEG motor imagery tasks to those of a cohort of severely brain-injured patients ($n = 28$) with a range of injury aetiologies, locations, and extents. As expected from previous work (Owen *et al.*, 2006; Monti *et al.*, 2010; Bardin *et al.*, 2011; Goldfine *et al.*, 2011), signals indicating covert cognition were found in many patients using both functional MRI and EEG. We focus on the spatial and temporal aspects of these signals and how they compare with those observed in healthy controls, and on the impact of arousal fluctuation on the accuracy of individual assessments. We discuss the implications of our findings in the context of challenges and opportunities for moving forward from evidence of command-following to establishing methods of interactive communication utilizing such signals.

Materials and methods

Participants

We studied 28 patients drawn from a larger sample enrolled in a multi-modal behavioural and imaging study of recovery from severe, non-progressive brain injury (21 males, seven females, age range at time of injury: 12–53 years, mean age at time of injury: 26.1 years, mean age at time of assessment: 31.6 years). All inclusion and exclusion criteria for enrolment in the larger study are detailed in the Supplementary material. We selected patient datasets for inclusion in the current study on the basis of data quality as well as consistency in the stimulus paradigms and data collection methods used. Sixty-four per cent of patients suffered from traumatic brain injury (TBI) while 36% suffered from other forms of injury (subarachnoid haemorrhage, trauma with haemorrhagic stroke, TBI with hypoxic ischaemia, vascular, hypoxia or anoxia). In addition, we obtained longitudinal studies in six patients at two time points. Diagnoses across the patient sample ranged from vegetative state to emerged from minimally conscious state as measured by a standardized behavioural assessment exam, the Coma Recovery Scale – Revised (CRS-R) (Giacino *et al.*, 2004). We confirmed all diagnoses upon admission to the study and obtained repeated CRS-R measurements during assessment periods. Patient information is detailed in Table 1.

We enrolled 15 healthy control volunteers in the study (seven males, eight females, age range: 23–55 years, mean age: 40.0 years). All controls had no history of neurological disease. Studies described herein were approved by the Weill Cornell Medicine and The Rockefeller University Institutional Review Boards. Controls gave their written consent. Consent was obtained for patients from their legally authorized representatives.

EEG experimental design

The experimental design and analysis methods used here were adapted from previously published methods (Goldfine *et al.*, 2011), which we summarize here for the reader's convenience. While undergoing video-EEG recording, patients and controls completed multiple trials of four different motor imagery tasks. We placed disposable earbud headphones in the subjects' ears and played a pre-recorded audio prompt for the duration of each trial. The prompt for each paradigm consisted of two commands played in succession, one to perform the requested task and the second to stop performing the requested task. Commands provided to participants are detailed in the Supplementary material. The four different motor imagery tasks included 'tennis' (swinging a tennis racket with one hand), 'open/close right (left) hand', 'navigate' (walking through one's house), and 'swim'. Most patients also completed a version of the 'open/close right (left) hand' paradigm that prompted them to actually perform the motoric action of opening and closing one hand. We instructed patients to try to perform the motion even if they were incapable of doing so. Control subjects only completed the version prompting them to imagine performing the action.

With the exception of two patients [Patient PS-10 (Assessment 2) and Patient PS-20], all runs of command-following tasks for patients were counterbalanced with other paradigms over the course of a multi-day study period. A failure to complete testing of all tasks was due to limitations on available study time secondary to clinical care needs.

A sequence of the two commands for a specific paradigm alternately repeated eight times, each constituted a 'run' and subjects completed multiple runs of each paradigm. Prior to each run, we instructed subjects to perform the requested motor imagery or motor task from the time they heard the 'task' command to the time that they heard the 'rest' command. We obtained verbal confirmation of task completion from all control subjects and some patients (if capable) after each run.

Each command was between 2 and 4 s in duration, and was followed by a silent response period that was at least 10 s in duration. To prevent including a non-specific response to the auditory stimulus itself in the analysis, the analysed response period commenced 1 s after the end of each command. Based on feedback from healthy controls that reported difficulty maintaining motor imagery for longer than several seconds, we analysed only the period between 1 and 10 s after the end of each command, resulting in a 9-s response period. The silent response period following each command varied in length between 10 and 14 s across some datasets and paradigms; however, the above-described structure of a command, followed by a 1-s buffer and then a 9-s analysed response period, was identical for every dataset.

EEG data acquisition

The EEG was recorded with 37 electrodes (Nihon Kohden collodion-pasted Ag/AgCl cup electrodes, 1.5 mm) arranged via an augmented 10-20 system with 18 additional electrodes (Jasper, 1958; Forgacs *et al.*, 2014). For Patient PS-10 (Assessment 1) only, EEG was recorded with 29 electrodes arranged via an augmented 10-20 system with 10 additional electrodes. For Patient PS-17 only, EEG was recorded with 23 electrodes via an augmented 10-20 system with four additional electrodes. The online EEG reference electrode was FCz for all recordings. Signals were recorded, digitized, and amplified using a Natus XLTEK EEG data acquisition system with one of the following headboxes: FS128, MOBEE32, or EMU40 (200, 250 or 256 Hz sampling frequency, impedance $\leqslant$ 5 kΩ, bandpass filters: 0.5 Hz and 70 Hz, notch filter: 60 Hz). Time-locked video of the subject during paradigms was recorded and Presentation software (Neurobehavioral Systems, Inc., Albany, CA) was used for command delivery and time-locking commands to the EEG record via an auxiliary channel of the EEG system.

EEG signal processing and artefact removal

We used detrended EEG from each 9-s response period and cut signals into 3-s epochs, yielding 24 total epochs for each condition per run. To avoid inclusion of runs during which the participant fell asleep in our analysis, we implemented a two-step screening process. First, during data collection, a trained clinical neurophysiologist (M.M.C.) made a determination of the participant's level of alertness prior to the initiation of any paradigm on the basis of behavioural cues and the presence or absence of EEG features of sleep physiology including spindles, delta waves, K complexes, and vertex waves. Second, following data collection but prior to data analysis, a fellowship-trained clinical neurologist (P.B.F.) reviewed the entire EEG record for each study and demarcated, based on the identification of the EEG features described above, any time points during which the participant was asleep; we then verified that these periods of sleep did not include any of our runs. Using this screening process, we excluded one patient run from analysis on the basis of sleep.

We then pruned signals via visual inspection and rejected epochs, and in some cases entire runs, contaminated by electromyogenic, eye-blink, or electrical interference artefact from

Table 1 Patient information and summary of EEG, functional MRI, and behavioural assessment results

Patient ID	Sex	Injury aetiology	Age at time of injury (y)	Age at time of assessment (y)	EEG command-following positive	Positive EEG paradigms/ EEG paradigms conducted, *n*	FMRI command-following positive	EEG background classification[a]	CRS-R Communication subscale score	Highest CRS-R score	Lowest CRS-R score	CRS-R exams, *n*	Diagnosis per CRS-R
Patients with positive EEG command-following during one or more assessments ($n = 21$)													
PS-1 (A1)	M	TBI	19	23	No	0/3	Yes	1	1	5	N/A	1	MCS
PS-1 (A2)	M	TBI	19	27	Yes	1/5	AC	1	1	5	N/A	1	MCS
PS-2	F	Anoxia	17	19	Yes	1/3	NT	2	0	6	5	2	VS
PS-3	M	TBI	20	23	Yes	1/3	No	2	0	6	2	4	VS
PS-4 (A1)	M	TBI	16	24	No	0/5	No	1	0	9	4	4	MCS
PS-4 (A2)	M	TBI	16	25	Yes	3/4	Yes	1	0	7	7	2	VS
PS-5	F	TBI	46	59	Yes	1/3	No	1	0	9	6	5	MCS
PS-6	M	TBI	17	47	Yes	1/3	NT	1	0	10	8	3	MCS
PS-7	M	TBI	22	25	Yes	2/3	Yes	2	0	11	8	3	MCS
PS-8	F	Vascular	22	28	Yes	1/4	No	1	1	12	7	4	MCS
PS-9 (A1)	F	TBI + hypoxic ischaemia	12	23	Yes	2/4	No	1	0	12	10	5	MCS
PS-9 (A2)	F	TBI + hypoxic ischaemia	12	26	No	0/4	No	1	0	13	12	3	MCS
PS-10 (A1)	M	TBI	23	25	Yes	1/1	Yes	1	2	16/17[b]	N/A	1	EMCS
PS-10 (A2)	M	TBI	23	29	Yes	3/3	No	1	2	23	N/A	1	EMCS
PS-11 (A1)	M	TBI	21	27	Yes	1/4	No	1	1	17	11	5	EMCS
PS-11 (A2)	M	TBI	21	28	Yes	3/3	No	1	0	14	12	3	MCS
PS-12	M	Anoxia	17	21	Yes	2/5	No	1	1	16	15	4	MCS
PS-13	M	TBI	50	55	Yes	1/3	No	2	0	17	11	5	EMCS
PS-14	M	TBI	19	25	Yes	1/4	Yes	1	0	17	13	4	MCS
PS-15	M	TBI	21	22	Yes	1/3	Yes	1	1	17	16	2	MCS
PS-16	M	TBI	18	35	Yes	1/5	NT	1	1	19	19	4	EMCS
PS-17	F	SAH with vasospasm	41	51	Yes	3/3	NT	1	1	21	20	2	MCS
PS-18 (A1)	F	TBI	17	19	No	0/3	Yes	1	0	10	9	6	MCS
PS-18 (A2)	F	TBI	17	20	Yes	1/3	No	1	2	22	21	2	EMCS
PS-19	M	SAH	53	56	Yes	3/3	NT	1	2	22	19	2	EMCS
PS-20	M	TBI + haemorrhagic stroke	20	32	Yes	1/4	Yes[c]	2	2	23	21	4	EMCS
PS-21	F	Anoxia	51	51	Yes	2/4	Yes	1	2	23	22	2	EMCS
Patients with indeterminate or negative EEG command-following during all assessments ($n = 7$)													
PS-22	M	TBI	32	38	No	0/3	No	1	0	4	2	2	MCS
PS-23	M	Anoxia	44	47	No	0/2	No	3	0	5	4	3	VS
PS-24	M	TBI	24	26	No	0/3	No	2	0	6	6	3	MCS
PS-25	M	TBI	22	27	No	0/3	No	1	0	10	5	9	MCS
PS-26	M	TBI	15	21	No	0/5	No	2	0	10	10	2	MCS
PS-27	M	TBI	17	27	No	0/3	No	1	0	11	9	2	MCS
PS-28	M	Hypoxia	36	39	No	0/3	No	2	1	15	14	2	MCS

[a]EEG background classification (1 = normal/mildly abnormal, 2 = moderately abnormal, 3 = severely abnormal).
[b]Motor function scale data missing in record.
[c]Distinct functional MRI protocol, see Rodriguez Moreno *et al.* (2010).
AC = artefact contaminated; A1 = Assessment 1; A2 = Assessment 2; EMCS = emerged from minimally conscious state; F = female; FMRI = functional MRI; M = male; MCS = minimally conscious state; NT = not tested; SAH = subarachnoid haemorrhage; TBI = traumatic brain injury; VS = vegetative state.

analysis. A single investigator (W.H.C.) completed all manual pruning to ensure consistency across runs, as well as conducted all EEG command-following analyses. Manual pruning resulted in comparable amounts of task and rest signal rejected for each run: on average, 64.9% of epochs for healthy controls and 56.4% of epochs for patients. The cleaned signals from each condition were concatenated separately, converted to the Hjorth Laplacian montage in order to increase ability to localize the sources of recorded signals, and band-pass filtered between 1–50 Hz (Hjorth, 1975, 1980; Thickbroom *et al.*, 1984). We performed all data processing offline in MATLAB (The Mathworks, Natick, MA) using a combination of EEGLAB, the Chronux toolbox, and in-house software (Delorme and Makeig, 2004; Bokil *et al.*, 2010; Goldfine *et al.*, 2011).

EEG spectral and statistical analysis

We calculated power spectral density estimates for each 3-s epoch using the multi-taper method via an implementation of the mtspectrumc code from the Chronux toolbox (Thomson, 1982; Percival and Walden, 1993; Bokil *et al.*, 2010). We utilized five tapers in our analysis, yielding a frequency resolution of 2 Hz and estimates spaced 1/3 Hz apart. We then averaged together spectra generated from the same run in response to the same command, respectively.

To identify differences in the frequency content of signals recorded in response to task or rest commands, we implemented a z-statistic, the Two Group Test (TGT), with a cut-off of $P \leqslant 0.05$ by jackknife method on a frequency-by-frequency basis for each run via an implementation of the Chronux toolbox code, two_group_test_spectrum (Bokil *et al.*, 2010).

Identification of EEG responses in controls

We queried controls upon the completion of each run in order to obtain verbal confirmation of task performance. We then applied the TGT to identify significant spectral differences between conditions on a frequency-by-frequency basis in channels not contaminated by artefact. Frequency spans smaller than 2 Hz are correlated as a result of our choices of multitaper function parameters and thus, we only considered spectral differences spanning two contiguous Hz or more to be significant. Spectral differences spanning < 2 Hz represented only a trend towards significance.

Identification of EEG responses in patient subjects

As we could not verbally confirm task performance in most patients, we applied more stringent measures to identify significant spectral differences between conditions. We used the two outcome measures previously published by Goldfine *et al.* (2011) to identify positive task performance in patients.

The first outcome measure required statistical significance as identified by the TGT ($P \leqslant 0.05$) as well as consistency in responses across runs. Fulfilment of the first outcome measure indicated a significant spectral difference between conditions spanning at least 2 contiguous Hz in at least one run and a trend towards significance, in the same channel and frequency range, in a different run of the same paradigm. However, the first outcome measure alone was susceptible to false-positive findings due to the problem of multiple comparisons.

For this reason, the second outcome measure accounted for multiple comparisons through false discovery rate (FDR) correction (Benjamini and Hochberg, 1995; Benjamini and Yekutieli, 2001). For each paradigm, we concatenated cleaned signals from all runs for each condition separately. We then calculated power spectra for each all-runs-combined signal and implemented the TGT ($P \leqslant 0.05$), with correction for a FDR of 0.05, to identify spectral differences. We rejected channels substantially contaminated by artefact from FDR correction analysis. The second outcome measure was fulfilled if, when all runs of one paradigm completed by a patient were combined, at least one significant spectral difference identified by the TGT remained significant after FDR correction among all channels and frequencies tested.

'Positive' task performance was defined as fulfilment of both outcome measures. If only the first outcome measure was met, performance was deemed 'indeterminate'. Fulfilment of neither outcome measure characterized 'negative' task performance (Goldfine *et al.*, 2011).

Estimation of EEG false positive results in patients

We performed an additional analysis of all 26 tennis datasets recorded in patients in order to estimate the rate of positive outcomes that would result from analysis of random EEG data using our methods. We focused our analysis solely on tennis task datasets because we had a maximal number of datasets for this paradigm. Additionally, we expected overall noise and signal characteristics to be comparable to those present in datasets from other paradigms in the same patient.

For each run, we randomly exchanged epochs between conditions to generate a surrogate dataset in which the original 'task' and 'rest' epochs were replaced by randomly relabelled epochs from the original dataset. The relabelling process was created by random exchanges that conformed to the following criteria: (i) only epochs from the same trial ('task' command followed by 'rest' command) were exchanged, in order to preserve the longitudinal integrity of the run; and (ii) the conditions in the surrogate runs each contained an approximately equal number of the original 'task' and 'rest' epochs (an imbalance of no more than two). We then applied our methods and outcome measures, as described above, to each surrogate dataset.

Clinical EEG analysis

A fellowship-trained clinical neurologist (P.B.F.) not involved in command-following analyses visually screened all EEG recordings using methods previously published by our group (Forgacs *et al.*, 2014). For each patient assessment, we classified wakeful EEG background into one of four categories based on the degree of abnormalities observed: normal, mildly abnormal, moderately abnormal, or severely abnormal. We classified a wakeful EEG background as 'normal' if there was a posterior dominant rhythm (PDR) of 8 to 12 Hz, an amplitude difference of not more than 50% between hemispheres, along with the expected anteroposterior gradient (gradual increase in frequency and decrease in amplitude from posterior to frontal areas with dominant 13–40 Hz 'beta' activity over the frontal cortices), and no focal or hemispheric slowing. We designated an EEG background as 'mildly abnormal' if the PDR was asymmetric or mildly slowed (not lower than 7 Hz), if the anteroposterior gradient was not well organized, and/or if a mild degree of focal or hemispheric slowing was present (slowing into the 4–7 Hz 'theta' range but not into the < 4 Hz 'delta' range). The designation 'moderately abnormal' indicated a dominance of theta (4–7 Hz) PDRs and/or presence of a moderate degree of focal or hemispheric slowing (slowing mostly in the theta range with occasional delta range slowing as well). We defined severely abnormal EEG background as a dominance of delta (< 4 Hz) waves over most brain areas. Due to the small number of patient subjects with 'normal' wakeful EEG background organization, we combined 'normal' and 'mildly abnormal' categories for later analyses. For each assessment, we based grading on the most normal EEG background observed.

Functional MRI experimental design, data acquisition, and statistical analysis

We conducted functional MRI studies in the 23 patients that both tolerated the study and did not have contraindications for MRI. Data were acquired on a General Electric 3.0T Signa Excite HDx MRI system, a Siemens 3.0T TIM Trio MRI system, or on a Siemens 3.0T MAGNETOM Prisma MRI system. Patients completed one run each of the tennis and open/close right hand tasks as described previously. Using a general linear model, we determined the difference of blood oxygenation level-dependent (BOLD) signals between the task and rest conditions to be significant with a FDR of 0.05. One patient, Patient PS-20, underwent a different functional MRI scanning protocol, the details of which have been published separately (Rodriguez Moreno *et al.*, 2010).

Except for the latter patient, our analysis consisted of the following steps: using SPM12 (v. 6225) (Friston *et al.*, 2008), we performed motion correction, slice-timing correction for interleaved acquisition, co-registration to the ICBM-MNI standard space EPI template (Mazziotta *et al.*, 2001), and spatial smoothing with an 8 mm filter. The general linear model was specified with a haemodynamic response function made of two gamma functions, which was convolved with the block design, an AR(1) model autocorrelation correction, the six motion parameters as nuisance regressors, and a constant for the intercept. The resulting statistical parametric maps were visualized with xjview (http://www.alivelearn.net/xjview) and an FDR threshold of 0.05 was applied.

In designing our analyses, we considered the possibility that patients with disorders of consciousness have the potential to demonstrate variable BOLD responses. Therefore, we considered a statistically significant response to the task all BOLD activations with corresponding $|t| > 3.1$. Of note, although past studies have demonstrated negative BOLD responses during motor tasks in healthy individuals (Allison *et al.*, 2000; Liu *et al.*, 2011), negative BOLD responses have not been characterized in the context of motor imagery. A single investigator (H.U.V.) conducted all functional MRI experiments and analyses, and was not involved in any other components of the study.

Results

EEG command-following in healthy controls

We observed EEG evidence of motor imagery task performance in all 15 healthy controls; 12 controls demonstrated a positive response to all paradigms tested, two controls demonstrated a positive response to three out of four paradigms tested, and one control subject demonstrated a positive response to two out of four paradigms tested. Temporal and spatial response characteristics varied only modestly across control subjects. A typical example of temporal characteristics during swim task performance in a healthy control—both alpha (8–12 Hz) and beta (13–40 Hz) spectral power suppression—is shown in Fig. 1A (HC-14; Laplacian derived channels Cz and O2 shown as examples). Figure 1B illustrates a summary of all TGT-identified significant spectral differences between task and rest conditions for each EEG channel and across all frequencies tested. In this subject, we observed alpha and/or beta spectral power suppression in the majority of channels (30 of 37).

Most healthy controls demonstrated spectral power suppression in the alpha (8–12 Hz) and/or beta (13–40 Hz) frequency ranges during task performance. As an example, 82.0% of significant responses observed in channel Cz during the tennis paradigm and 86.7% of significant responses observed in channel Cz during the open/close right hand paradigm demonstrated suppression of spectral power in either the alpha or beta range, or both. Descriptions of all positive healthy control responses to all paradigms are detailed in Supplementary Table 1.

Control subjects also demonstrated a spatial consistency of statistically significant responses. Figure 2 illustrates the locations, by channel, of significant power spectral differences between conditions (task and rest) for healthy control runs (left column) and patient subjects (right column). Response profiles, plotted here as topoplots, were derived from pooled subject responses to the individual paradigms; the percentage of individual runs (for healthy controls) or patient subjects with significant power modulation in each channel is indicated by the colour bar. For healthy controls, there are clusters of consistently significant EEG channels, indicated as hot colours, for all paradigms. We designated the individual channels with the consistently highest response percentages in each control profile as 'channels of interest', which we use as a basis for comparison with patient responses below. These channels of interest are indicated by the labelled blue dots on the control profiles and the same channels are also indicated by white circles on the corresponding topoplots for patients.

Generally, across control subjects, there was an overlap in the spatial response patterns across paradigms; for example, channel CP1 was identified as a channel of interest for all paradigms. However, differences among the

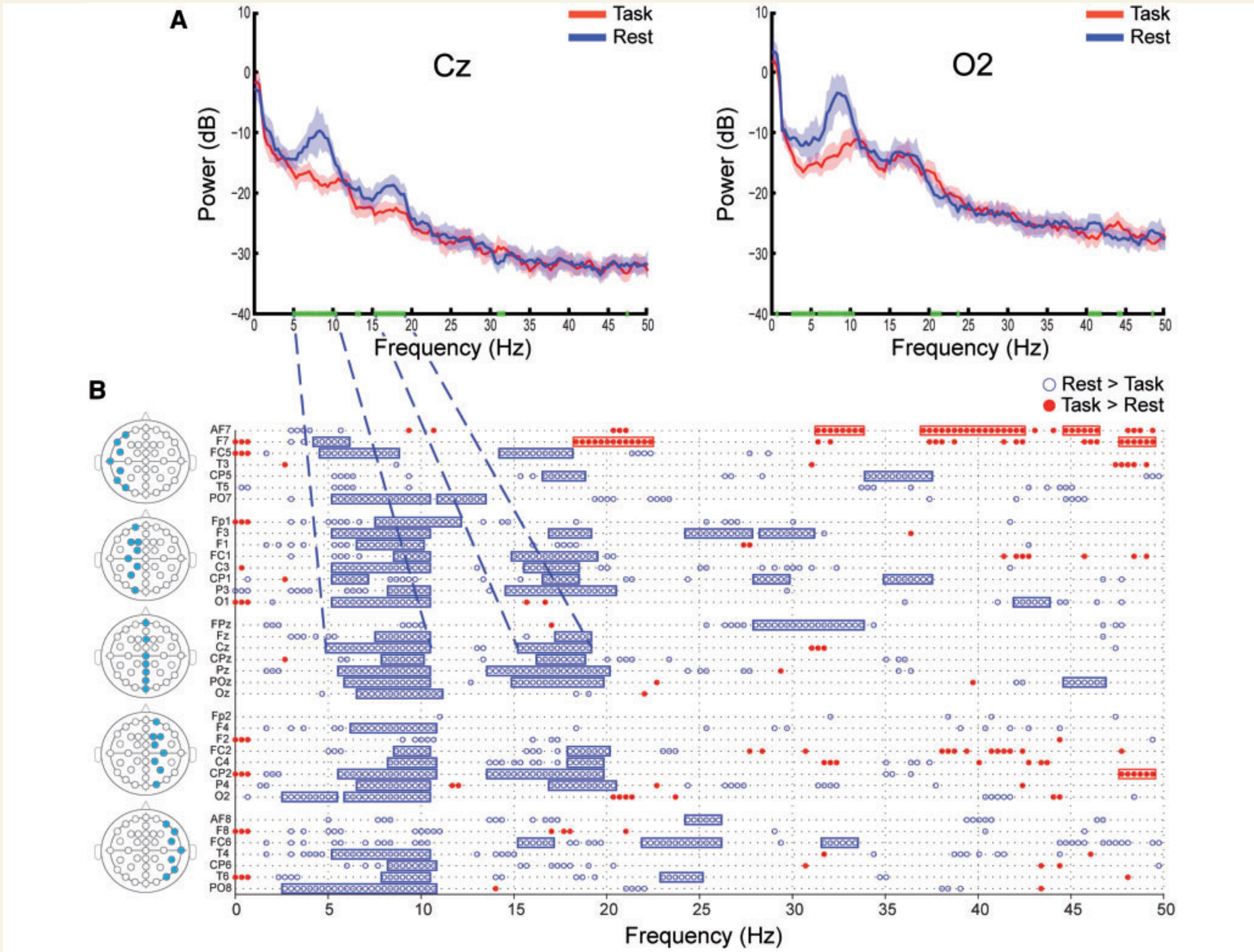

Figure 1 Example of significant, diffuse alpha (8–12 Hz) and beta (13–40 Hz) spectral power suppression during 'swim' task performance in healthy control subject HC-14. (**A**) Power spectral density estimates from EEG channels Cz and O2 during task (red) and rest (blue) conditions. Green stars along the *x*-axis designate TGT-identified significant differences in power between conditions ($P \leqslant 0.05$). (**B**) Summary of TGT-identified significant differences between conditions for all channels and frequencies tested. Red circles signify frequencies at which power was greater during task performance relative to rest. Blue circles signify frequencies at which power was greater during rest relative to task performance. Rectangles designate significant differences in power that spanned 2 Hz or more, thus meeting our threshold for statistical significance. Channels are grouped according to electrode location on the scalp. 27.82% of TGT-identified values remained significant after FDR correction (0.05) (not shown).

response topographies corresponded with unique aspects of each task. For 'swim', a task involving imagination of a full-body motion, our results showed a bilateral pattern of responses in frontocentral (FC2), central (Cz, C4), and centroparietal (CP1, CPz, CP2, Pz) sensorimotor regions. For 'tennis' (right hand), we observed a broader pattern of responses in addition to an enhanced pattern of power modulation in the contralateral hemisphere over channels centred on the motor cortex and hand representation region (C3, CP1). The 'open/close right hand' profile demonstrates a localized and lateralized concentration of responses over the motor strip (Cz, C3, CP1) and the lack of a frontocentral (FC1, FC2) component seen in other profiles. In contrast to the other tasks, 'navigate' gave rise to a sparser topography with more posterior region responses. The corresponding response topography, while containing frontocentral (FC2) and centroparietal (CP1) features consistent with the other profiles, lacked a tight spatial concentration of responses and revealed a pattern of posterior temporal (T6) and parietal (PO7) power modulation not seen in the other three profiles. Additionally, the 'navigate' response profile was generated from fewer runs relative to the other paradigms, which could account for the sparseness of the topography.

EEG and functional MRI command-following outcomes in patients

Twenty-one of 28 patients studied demonstrated a positive outcome for a minimum of one EEG paradigm, during at least one assessment. All other patients demonstrated either

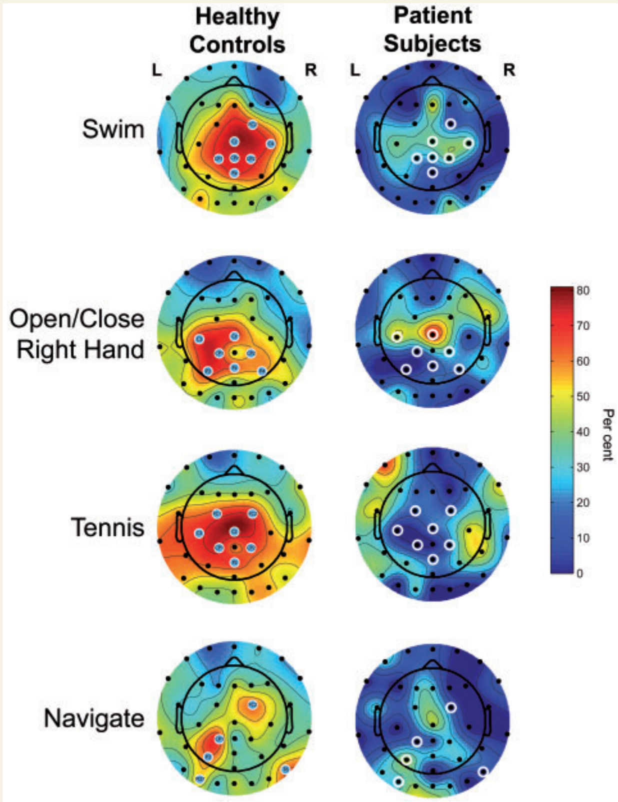

Figure 2 Response profiles. *Left*: Response profiles for individual paradigms shown as topoplots of the percentage of individual control runs with significant power modulation in each channel, across subjects ('swim': 18 healthy control runs, 'open/close right hand': 21 healthy control runs, 'tennis': 27 healthy control runs, 'navigate': 13 healthy control runs). Percentages for each channel are indicated by the colour bar. Each profile demonstrated a small number of channels with consistently high response percentages across runs, designated as 'channels of interest', which are shown as blue circles on the control profiles. *Right*: Response profiles for the same four paradigms shown as topoplots of the per cent of patients demonstrating a positive response in each channel ('swim': eight patients, 'open/close right hand': 10 patients, 'tennis': six patients, 'navigate': seven patients). Control subjects completed the version of the 'open/close right hand' paradigm prompting them to imagine the motoric action, while patients completed the version of the paradigm prompting them to actually perform the motoric action. Unlike in the responses of controls, we did not observe a spatial consistency in the responses of patients. The channels of interest derived from the control profiles are designated by white circles on the patient profiles.

indeterminate or negative outcomes on all EEG and functional MRI paradigms tested. Table 1 summarizes these findings. For comparison, all patients with functional MRI evidence of command-following demonstrated a positive EEG command-following outcome during at least one assessment, indicating that our EEG approach is at least as sensitive as prior functional MRI methods (Monti *et al*., 2010; Bardin *et al*., 2011). Seven of the 21 patients with demonstrated EEG evidence of command-following did not show evidence of command-following as detected by functional MRI during any assessment. Importantly, all five patients that could not tolerate functional MRI testing because of ferromagnetic implants or other factors in this cohort did demonstrate EEG evidence of command-following. EEG command-following analysis findings aligned with functional MRI findings for 13 patients. Two of the nine patients with functional MRI evidence of command-following [Patient PS-4 (Assessment 2) and Patient PS-21] demonstrated significant, negative BOLD responses. Five of the patients studied longitudinally demonstrated changes in EEG and functional MRI findings between assessments. Table 1 details the relationships among different testing outcomes in the patients studied longitudinally with inconsistent test-retest findings.

Table 1 also shows patient demographics and highest CRS-R total and communication subscale score recorded during each assessment. As shown in Fig. 3A, patients with EEG evidence of command-following capacity demonstrated a range of performance on the CRS-R. CRS-R scores for EEG responders ranged from 5 to 23 while scores for non-responders ranged from 4 to 15. All 12 patients with a CRS-R exam score higher than 15 during a particular assessment demonstrated EEG evidence of command-following during the same assessment, while only 4 of these 12 patients demonstrated functional MRI evidence of command-following during the same assessment. Additionally, 9 (42.9%) of the 21 patients demonstrating EEG evidence of command-following possessed no identifiable communication channel as detected with the CRS-R exam (CRS-R communication subscale score = 0). Nine patients demonstrated positive EEG performance of two or more tasks during at least one assessment; this patient subgroup exhibited no difference in total CRS-R scores relative to the patient responder cohort as a whole.

Patients with positive EEG evidence of command-following demonstrated variability with regard to which tasks they successfully performed. Most commonly, patients demonstrated a positive response to only one paradigm (57.1%), and 10 (47.6%) of 21 patients with EEG evidence of command-following only demonstrated a positive response to either the 'swim' task or one of the 'open/close hand' tasks. Only one patient (Patient PS-15) demonstrated a positive response solely to the 'tennis' task and two patients (Patient PS-1 and Patient PS-8) demonstrated a positive response solely to the 'navigate' task. All but one patient exhibiting a positive response to multiple tasks demonstrated positivity on either the 'tennis' task or the 'navigate' task. The different paradigms elicited positive responses in comparable numbers of channels across all patients. Although we were unable to conduct all paradigms in all patients, the clinical characteristics of patients that performed each task did not meaningfully differ on a group level (Supplementary Table 3).

Estimation of EEG false positive results in patients

We estimated the predicted rate of false positive outcomes that would result from analysis of surrogate datasets

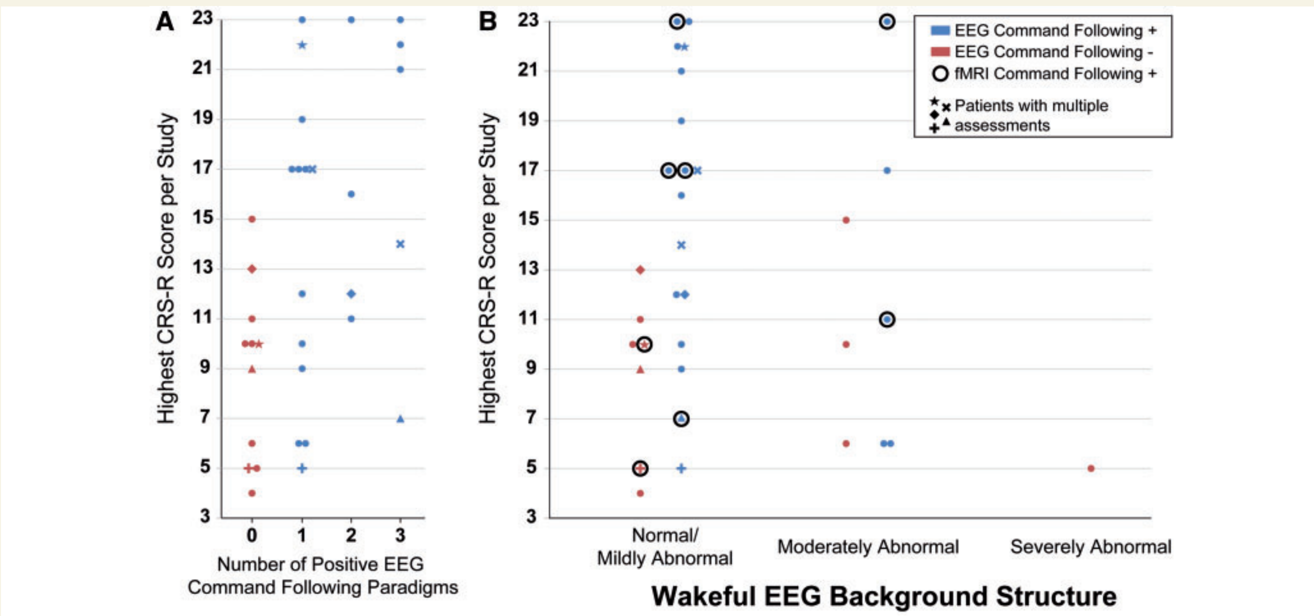

Figure 3 Total CRS-R score summary for all patients studied. Excluding Patient PS-10 (Assessment 1) because of missing data. (**A**) Highest recorded CRS-R score per assessment for both EEG command-following positive (blue) and EEG command-following negative (red) patients. Patients are organized according to the number of paradigms for which they demonstrated a positive outcome. Subjects with two assessments shown are designated by the alternate symbols and both assessments are plotted. (**B**) Patients separated into three classifications of wakeful background EEG activity. Patients with normal and mildly abnormal background EEG are grouped into the same category. Black circles designate patients with functional MRI (fMRI) evidence of command-following.

containing randomly shuffled EEG data recorded in patients using our methods and outcome measures. Analysis of surrogate datasets yielded a positive outcome in 2 (7.7%) of 26 of patient 'tennis' datasets tested (see 'Materials and methods' section).

Signal characteristic variability in patients

Figure 4 shows distinct examples of positive EEG 'tennis' task performance in two different patients (Patient PS-19, Fig. 4A and Patient PS-9, Fig. 4B). As shown in Fig. 4A, a broad elevation of ~10–20 Hz power associated with task performance in Patient PS-19 as well as a more localized suppression of ~20–30 Hz power in the right posterior temporal-parietal region. By contrast, performance of the same task in Patient PS-9 was characterized by a constrained suppression of parietal low beta (~16–18 Hz) power along with a combination of beta power elevation and suppression in frontocentral, parietal, and temporal regions (Fig. 4B). Descriptions of all positive patient responses to all paradigms are detailed in Supplementary Table 2.

A comparison of the distribution of frequencies of responses to 'open/close right hand' within channel Cz in the healthy controls compared to the patients revealed a strong consistency only in the controls, with 86.7% of control runs containing either suppression of alpha (8–12 Hz) or beta (13–40 Hz) spectral power, or both. No patient with a positive response to the same paradigm demonstrated significant alpha spectral power suppression and 33.3% demonstrated only beta spectral power suppression.

We did observe a semblance of overall spatial consistency in the positive EEG responses of patients to each task in the context of the control response topographies (Fig. 2, left column). For each paradigm, at least 80% of patients demonstrating positive EEG task performance responded in at least one channel of interest ('swim': 87.5%, 'navigate': 85.7%, 'tennis': 83.3%, 'open/close right hand': 80.0%). On a more granular level, however, the spatial consistency of responses across control runs for each paradigm was not present in the responses of patients (Fig. 2). For this reason, we could not establish channels of interest from the patient response topographies. On average, 68.2% of control runs contained a significant response in a particular channel of interest while only 22.1% of patients exhibited a response in a particular channel of interest (Fig. 5). A notable exception to this distinction is the percentage of responses seen across both control runs and patients to the 'open/close right hand' task, in that very similar percentages of control runs (71.0%) and patients (70.0%) demonstrated a significant response in channel Cz. However, as described above, this instance of spatial consistency in patients was not mirrored by a consistency in reporting frequencies.

Clinical EEG in patients

We observed variability in wakeful EEG background characteristics across patient responders (Fig. 3B). Patients with EEG and/or functional MRI evidence of command-following

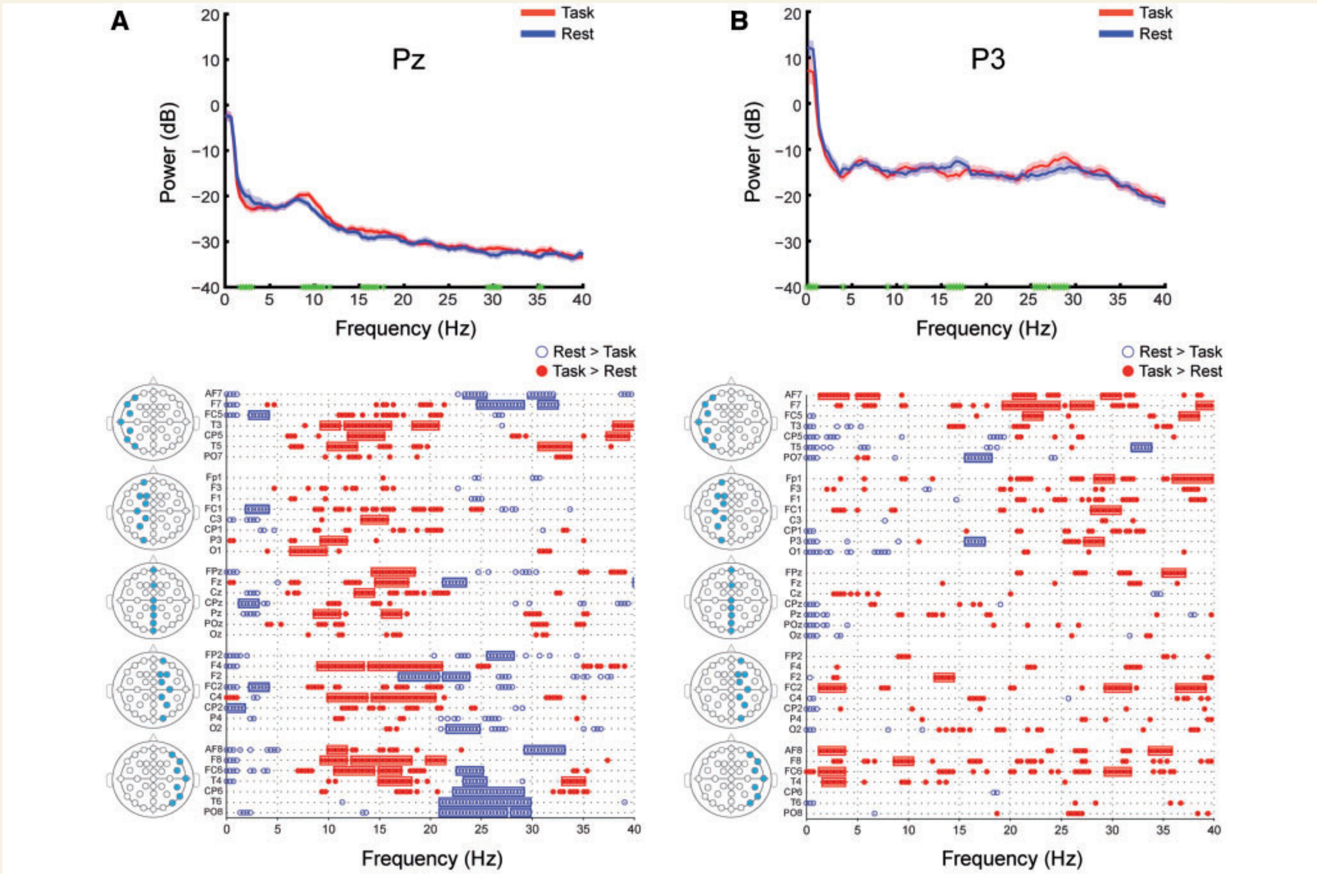

Figure 4 Two distinct examples of positive patient responses to the 'tennis' paradigm. (**A**) *Top*: Power spectral density estimates from channel Pz during task performance (red) and rest (blue) in Patient PS-19. Green stars along the *x*-axis designate TGT-identified significant differences in power between conditions ($P \leqslant 0.05$). *Bottom*: TGT summary plot generated from all runs of the 'tennis' task from Patient PS-19 combined. Rectangles designate significant differences in power that spanned 2 Hz or more. A broad elevation of alpha-low beta (~8–20 Hz) power—as seen in channel Pz—associated with task performance as well as a more localized suppression of ~20–30 Hz power in the right posterior temporal-parietal region. 3.61% of TGT-identified values remained significant after FDR correction (0.05) (not shown). (**B**) *Top*: Power spectral density estimates from channel P3 during task performance and rest in Patient PS-9 (Assessment 1). *Bottom*: TGT summary plot generated from all runs of the 'tennis' task from Patient PS-9 (Assessment 1) combined. A constrained suppression of parietal low beta (~16–18 Hz) power—as seen in channel P3—accompanied by a combination of beta power elevation and suppression in frontocentral, parietal, and temporal regions associated with task performance. 0.37% of TGT-identified values remained significant after FDR correction (0.05) (not shown).

demonstrated both 'normal or mildly abnormal' and 'moderately abnormal' wakeful EEG background activity. No patient with severely abnormal wakeful EEG background activity demonstrated evidence of command-following by either functional MRI or EEG assessment. Among the patients with moderately abnormal EEG background activity, only those with a CRS-R exam score of 11 or higher demonstrated functional MRI evidence of command-following, while two patients with a CRS-R exam score of 6 demonstrated EEG evidence of command-following.

State fluctuations in patients

We next asked whether the 'indeterminate' outcome in some patients could be a manifestation of fluctuations in state—namely, that responses were present in some runs but not in others. The following analysis was not intended to reclassify the performance of patients but rather to explore the prevalence of state fluctuations among patients in our cohort. We focused on the 'tennis' paradigm because it yielded the highest number of indeterminate responses.

Figure 6 shows an example of this state change analysis applied to Patient PS-18 (Assessment 1), for whom analysis of a subset group of runs gave rise to a positive result. To identify state fluctuations, we combined EEG segments from both task and rest conditions recorded during each run of the paradigm to generate a single, composite power spectrum for each individual run (Fig. 6A). The composite spectrum provided an indicator for the overall shape of the power spectrum present across the time elapsed of each individual run. We overlaid the spectra for each individual run and used visual inspection of each channel within the predetermined set of 'tennis' channels of interest (Fig. 2) to look for spectral features arising across the combined runs

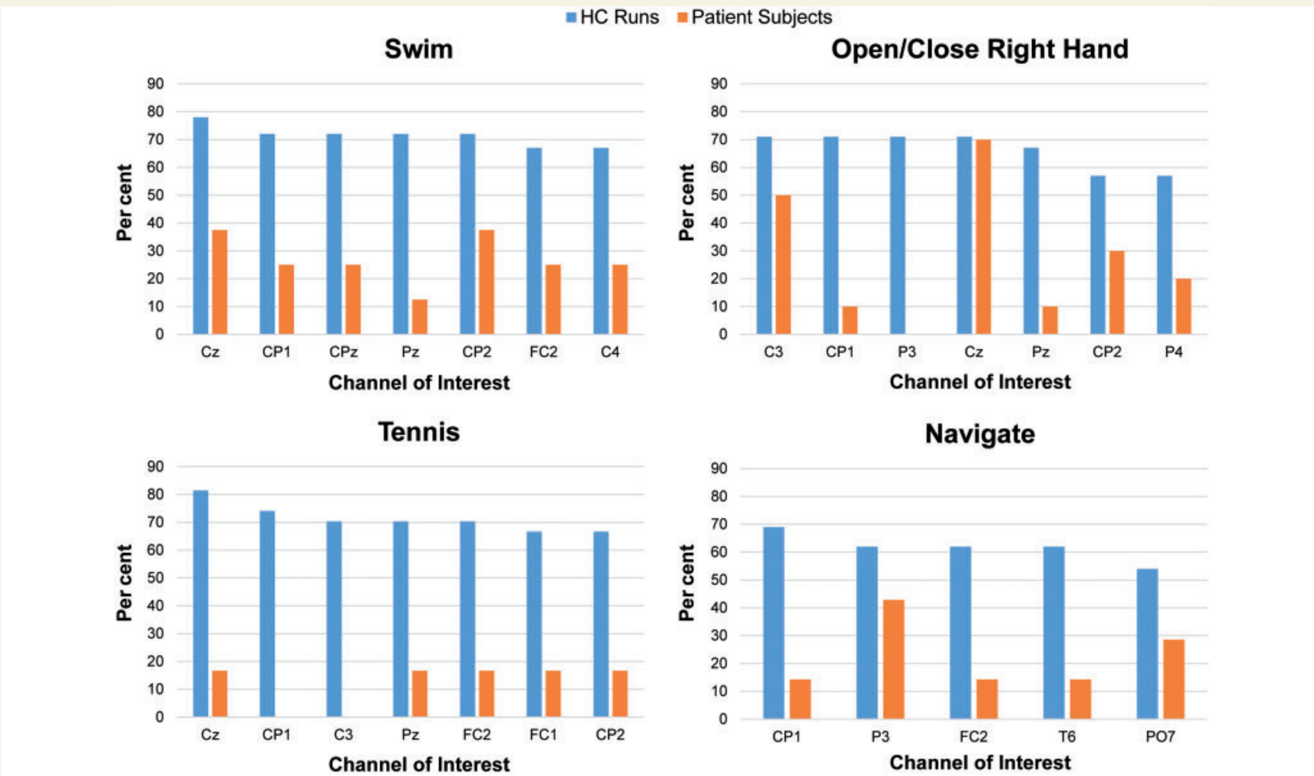

Figure 5 Frequency of channel of interest responses by paradigm. Histograms illustrating the per cent of positive patient (orange) and control runs (blue) with a significant response in each channel of interest, for the four paradigms completed by controls ('swim': 18 control runs, eight patients; 'open/close right hand': 21 control runs, 10 patients; 'tennis': 27 control runs, six patients; 'navigate': 13 control runs, seven patients). Control subjects completed the version of the 'open/close right hand' paradigm prompting them to imagine the motoric action, while patients completed the version of the paradigm prompting them to actually perform the motoric action. Responses from each group to the respective paradigm are plotted on the same graph. HC = healthy control.

that separated the runs into groups (Fig. 6B and C). If a state change appeared to be present by visual inspection of the shape of the power spectra, we then segregated runs into groups based on similarity of spectral features and applied our formal statistical measures to each new grouping of runs. Of 18 patients demonstrating indeterminate 'tennis' task performance, nine exhibited fluctuations in state between individual runs of the paradigm, as evidenced by global changes in power spectrum features similar to those shown in Fig. 6A. For five of these nine patients, independent analysis of a subset of all recorded runs gave rise to a positive result per our statistical measures. All of these five patients, however, had shown at least one positive response to a different paradigm.

To follow these methods, we then attempted to apply an objective, non-visual inspection measure to classify runs based on spectral shape. To do this, we first calculated the mean power spectrum, for each run, of the seven 'tennis' channels of interest. We then calculated the mean power, of this mean spectrum, from 1.0–7.0 Hz and from 7.3–20.0 Hz for each individual run, yielding two values for each run. We plotted the mean power from 1.0–7.0 Hz against the mean power from 7.3–20.0 Hz for each run and grouped runs that clustered together. The groupings of runs resulting from this analysis aligned with the groupings based on visual inspection for seven of the nine patients demonstrating evidence of state changes based on visual inspection. However, since this method could not discriminate differences in spectral features between runs in all cases, we relied on visual inspection as our primary measure to identify fluctuations in state (data not shown).

Changes in EEG response characteristics across assessments in one patient subject

In addition to the observation of state fluctuations contained within a single assessment, we identified shifts in response characteristics to the 'swim' paradigm in the test-retest evaluation of one patient (Patient PS-10) across a 5-year time period. No other patient studied longitudinally demonstrated positive performance of the same task across both assessments. Patient PS-10's highest CRS-R score for the first assessment was 16 out of 17 (motor function scale data missing in record; patient quadriplegic), while the highest score for the second assessment was 23 out of 23. The patient was fitted with a head mouse controller during the first assessment. After ~2–3 years with the regular usage of the head mouse as a BCI, the patient

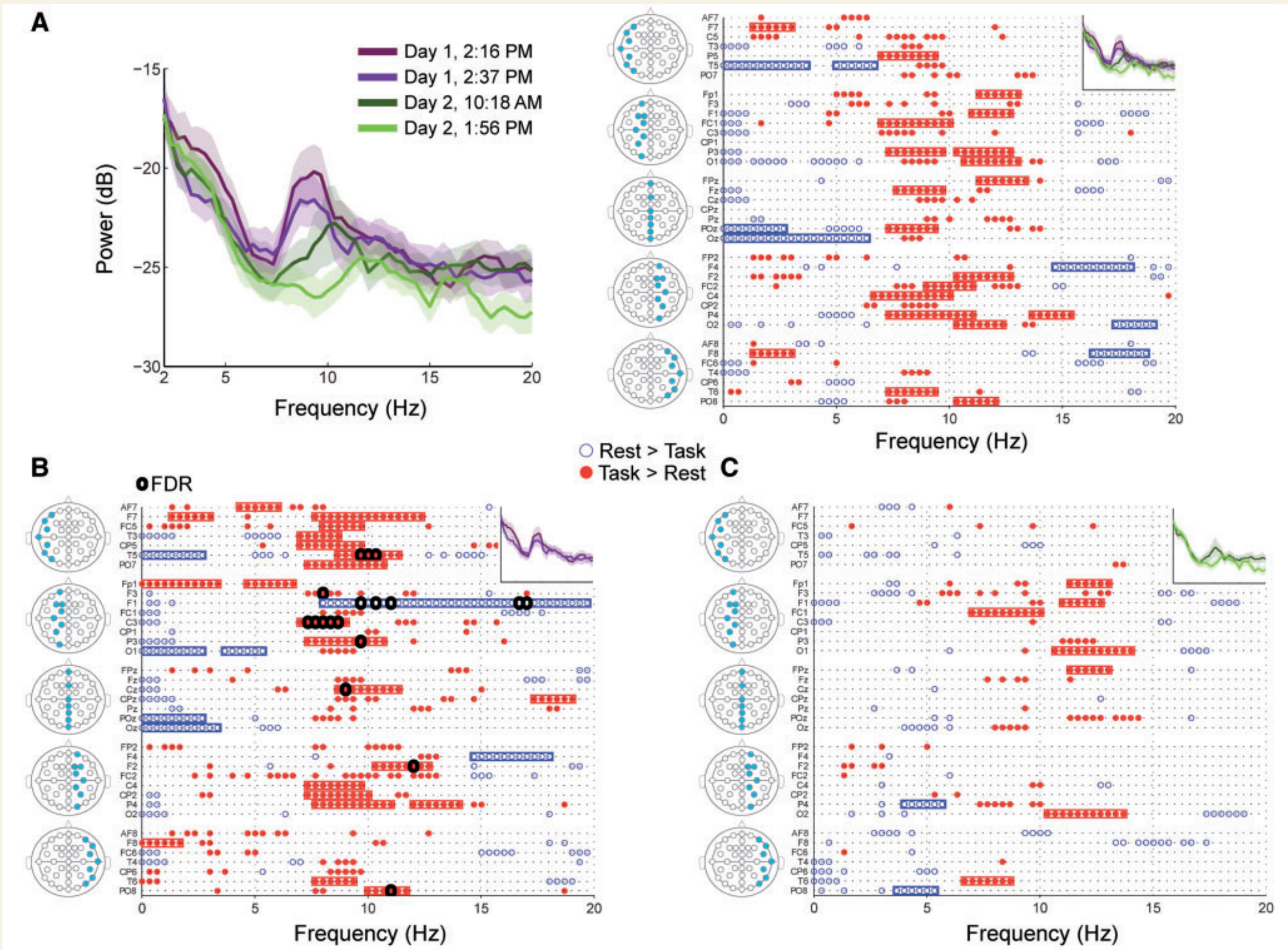

Figure 6 Example of state fluctuation analysis applied to runs of 'tennis' in Patient PS-18 (Assessment 1). (**A**) *Left*: Composite power spectra for four runs of 'tennis', generated from all cleaned EEG recorded from channel CP2 (one of the seven predefined 'tennis' channels of interest; see text) during task and rest conditions, for each respective run, combined. The composite spectrum for each run provided an indicator for the overall shape of the power spectrum present across the time elapsed of each individual run. The two runs from Day 1 (plotted in purple) demonstrate a distinct alpha peak feature, which is largely absent in Day 2 runs (plotted in green). *Right*: TGT summary plot obtained from analysis of all four runs of 'tennis' from Patient PS-18 (Assessment 1)—the spectra *inset* (*top*) indicates the group of runs (in this case, all four) on which the analysis was performed. When we combined task and rest EEG from all four runs and applied our statistical criteria, no TGT-identified significant values remained significant after FDR correction (0.05). (**B**) TGT summary plot obtained from independent analysis of Day 1 runs only; 2.65% of TGT-identified spectral differences between conditions remained significant after FDR correction (0.05) and are designated by the black ovals. (**C**) TGT summary plot obtained from independent analysis of Day 2 runs only; no TGT-identified differences remained significant after FDR correction (0.05).

developed capacity to control a joystick using his left hand. As shown in Fig. 7A, the delta range (1–4 Hz, demarcated by the green rectangle) for the first assessment contains a mix of spectral power increase and suppression during task performance whereas during the second assessment (Fig. 7B), we only observed spectral power suppression in the same frequency range. Additionally, we observed significant spectral power suppression constrained within the alpha range (8–12 Hz, demarcated by the purple rectangle) during the second assessment but not during the first. The summary plot for the first assessment also shows a mix of spectral power increase and decrease during task performance in the beta range while we observed generalized beta spectral power decrease during task performance during the second assessment.

Discussion

In this study, we found a broad heterogeneity in patient-generated EEG responses to motor imagery command-following tasks. Although over 80% of positive patient responders exhibited significant responses within at least one of the channels of interest identified in the controls for all paradigms, as a group, patients showed considerable spatial variation in reporting EEG channels (Figs 2 and 5).

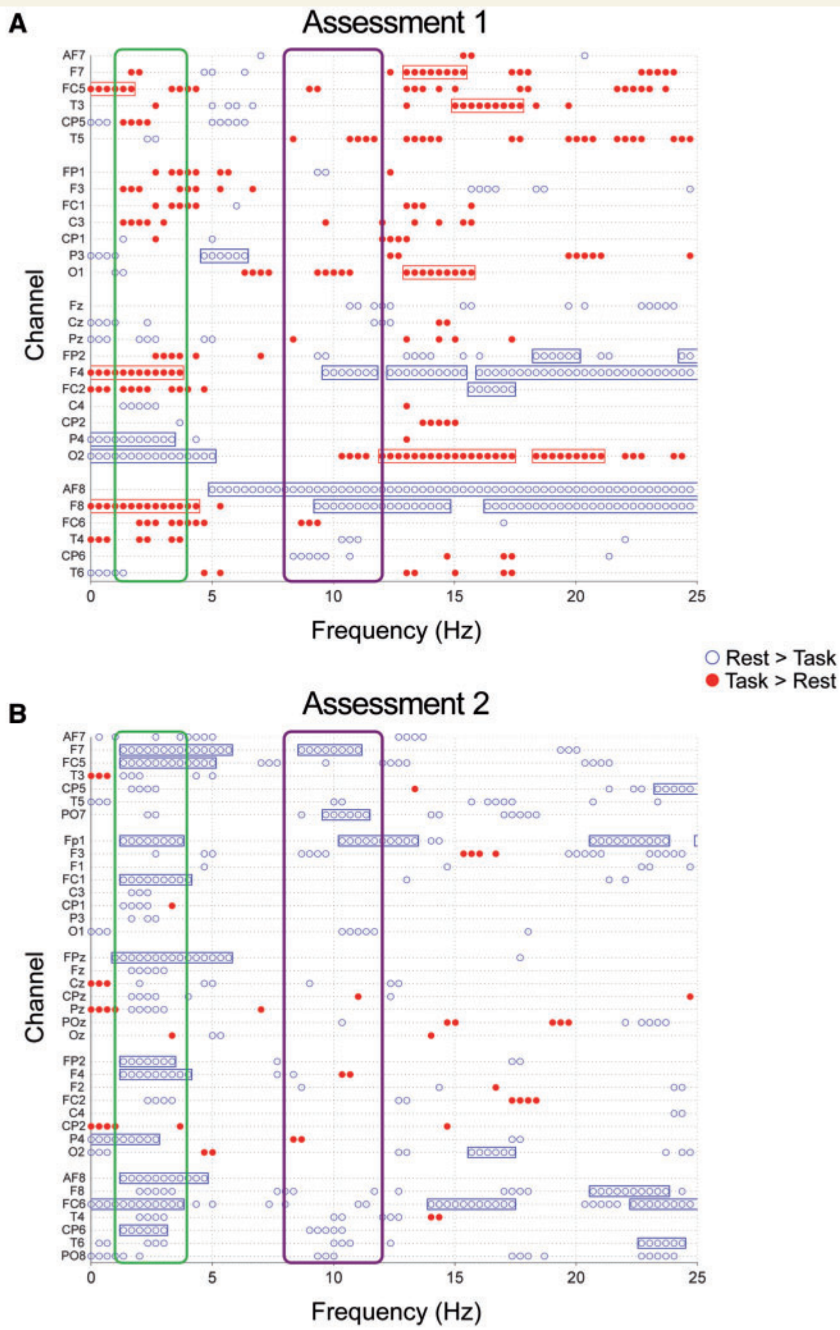

Figure 7 Changes in response signal over time in a patient. Patient PS-10 demonstrated a shift in response characteristics to the 'swim' task between assessments 5 years apart, before (**A**) and after (**B**) use of an independent BCI (head mouse controller). Green rectangles demarcate the delta range (1–4 Hz) and purple rectangles demarcate the alpha range (8–12 Hz). A mix of spectral power increase and suppression associated with task performance at the time of the first assessment (**A**) whereas only spectral power suppression was observed at the time of the second assessment (**B**).

In addition, we observed significant variability in the reporting frequencies across patient responses (Fig. 4 and Supplementary Table 2). Most commonly, patients with EEG evidence of command-following only demonstrated a positive response to one paradigm and 47.6% of all patient responders only exhibited positive responses to either one of the 'open/close hand' paradigms or the 'swim' paradigm. In total, EEG spectral analysis identified the capacity for command-following in 21 of 28 patients (75.0%), of whom 9 of 21 (42.9%) demonstrated no evidence of communication ability as measured with our standardized behavioural assessment tool (CRS-R communication subscale score = 0). Additionally, only 9 of 28 patients (32.1%) exhibited functional MRI evidence of command-following, supporting the added utility of electrophysiological detection. Collectively, a wide range of performance on the CRS-R as well as some variability in wakeful background EEG organization characterized the positive patient responders to EEG motor and motor imagery paradigms (Fig. 3). However, normal or mildly abnormal EEG wakeful background activity characterized the majority of responders. These findings are consistent with the inference that CMD is closer to locked-in state in terms of the overall integrity of the corticothalamic system (Schiff, 2015) and consistent with prior 24-h sleep-wake EEG studies that correlated positive functional MRI command-following with integrity of sleep-wake EEG architecture (Forgacs *et al.*, 2014). Sensitivity to fluctuations in arousal state occurring between EEG runs influenced assessment of outcome in half of all indeterminate responders tested on the 'tennis' task (Fig. 6). In some cases, these arousal fluctuations may have precluded our ability to detect a positive command-following response. Specifically, arousal fluctuations may have masked positive responses to the 'tennis' paradigm in up to five of the nine patients with observed fluctuations. Additionally, in one patient studied longitudinally at a 5-year interval, a marked shift in response characteristics to the same task was seen (Fig. 7). These observations present evidence of ongoing plastic changes in networks responsive to these EEG tasks and more generally, shed light on the expected physiological differences between CMD patients and healthy individuals.

Comparison of healthy controls and patients

Patient responses showed significant variability compared to those of control subjects. Patient responses demonstrated a diverse range of reporting frequencies in the power spectrum (Fig. 4 and Supplementary Table 2) while control responses largely demonstrated alpha and/or beta spectral power suppression during task performance (Fig. 1 and Supplementary Table 1). These findings are consistent with previous investigations of EEG motor imagery performance in healthy individuals (Pfurtscheller and Neuper, 1997; Pfurtscheller and Lopes Da Silva, 1999). Although some controls will show variation with respect to power increases or decreases in the alpha and beta ranges with motor imagery, low frequency modulation is not typically seen in this context (Bai *et al.*, 2008; Goldfine *et al.*, 2011). Regarding spatial characteristics, patients demonstrated variation in reporting EEG channels while controls demonstrated consistency in this domain (Figs 2 and 5). For example, across control responses to the 'tennis' (right hand) task, we consistently observed a pattern of EEG power modulation in the contralateral hemisphere over channels centred on the motor cortex and hand representation region (Fig. 2). Whole-brain functional MRI activation patterns using the same 'tennis' paradigm in healthy volunteers demonstrate activation in underlying cortical regions generally consistent with these findings (Boly *et al.*, 2007).

The marked differences in both spatial variation and frequency ranges of EEG modulation in the patient group compared to the controls may reflect specific pathophysiological mechanisms. Specifically, patient results show modulation of low frequency power, wide spatial variation in reporting channels, and a bias toward evidence of performance of only specific tasks. Across the multiple paradigms used here, the majority of patient responders (57.1%) only successfully performed one task and of those, nearly half of the responders only demonstrated a positive response to either the 'swim' task (23.8%) or one of the 'open/close hand tasks' (23.8%). These observations likely take origin in the relationship of brain regions supporting specific tasks, the relative cognitive load of a particular task for an individual subject, or the availability of viable reporting cortical regions. More generally, however, our data show a wide variance in the likelihood of any individual paradigm to yield positive results in a particular patient (*cf*. Gibson *et al.*, 2014).

The presence of low frequency power modulation in patient responses likely results from expected pathophysiology. Features of the EEG power spectrum reflect synchronous activity, and the presence of low frequency theta and delta features are expected based on varying patterns of deafferentation and multi-focal injuries in these patient subjects (Schiff *et al.*, 2014). Recruitment of widely distributed cognitive networks can be thought to draw some neuronal populations participating in low frequency oscillations at rest into the network computations by providing sufficient afferent drive to remove their contribution to resting theta or delta components of the EEG. Depending on a variety of possible effects on entrained oscillators, this may result in a sharpening of a low frequency oscillation or suppression of low frequency power in the delta or theta range. In patients, motor imagery task generation can thus result in signal characteristics not typically seen in healthy individuals (Fig. 4). Additionally, task-related desynchronizations in the delta range can perhaps be considered a marker for recruitment of cells that remain with subthreshold levels of activation but can nonetheless be recruited into participation in a large-scale cortical network supporting task performance. Our findings are supported by and similar to those of Edlow *et al.*, (2017) who observed delta range desynchronizations during a language comprehension task in DOC patients.

Other studies have used alternative methods, specifically, event-related potentials, to probe cognition in DOC (Gibson *et al.*, 2016) and some such studies have encountered similar heterogeneity across patients in the form of variable P3 latency (Hauger *et al.*, 2015). The overall lack of both a spatial or frequency consistency across patient responses to our paradigms is an important caveat for approaches that aim to detect patient responses by filters determined to capture responses of control subjects (Cruse *et al.*, 2011; Goldfine *et al.*, 2013). Since patient responses may occur in different locations and with different temporal characteristics than those of control subjects, such approaches can lead to false negatives. They are also at risk for false-positive responses, since patient EEGs may have greater overall levels of artefact.

A priori, the signal locations we observed in patients could have artefactually appeared to be more heterogeneous merely because some of these signals were false positives and arose at random locations. However, the surrogate analysis shows that this is unlikely; we found a positive outcome in only 2 of the 26 (7.7%) negative surrogates constructed from patient datasets. Removal of any two datasets from Fig. 2 would not have altered our conclusions about the spatial distribution of responses across patients.

The other marked distinction between control and patient EEG responses seen is the observation of state fluctuations in patients and what appears to be a covariation of EEG responses to command-following tasks (Fig. 6). Other studies have noted the potential impact of arousal fluctuations on the ability to detect evidence of cognition in this patient population during one-time recordings (Lule *et al.*, 2013; Hauger *et al.*, 2015). The sensitivity of some patients' EEG signal characteristics to changes in state is highlighted by our ability to find initially undetected evidence of command-following in over half of all subjects showing fluctuating arousal state across multiple runs of assessment. We are not advocating reclassification of such outcomes as positive because of the potential pitfalls of relying on visual inspection to group runs and the many ways in which subsets of runs could be chosen. Nonetheless, our results support the critical impact of arousal regulation on demonstration of cognitive capacities in patients with severe brain injuries (Schiff, 2010).

Such arousal dysregulation may take origin in impaired function of the anterior forebrain mesocircuit in patients with disorders of consciousness (Schiff, 2010, 2016; Fridman *et al.*, 2014). The mesocircuit model proposes that central thalamic excitation of the cortex is critical to the maintenance of conscious awareness (Schiff, 2010). Severe brain injury has been observed to result in downregulation of central thalamic activity, relative to controls, potentially due in part to increased globus pallidus inhibitory activity reducing outflow from an already deafferented and disfacilitated thalamus (Fridman *et al.*, 2014). Thus, the arousal fluctuations observed in some patients may be attributed to an inability to consistently maintain thalamocortical excitability due to injury-induced structural or metabolic constraints. Although every patient in this subsample who regained a positive response to 'tennis' after accounting for state fluctuations had shown at least one positive response to another paradigm, our findings raise the question of whether further testing over time might have revealed statistical evidence of command-following in the two non-responders who also exhibited fluctuations in state. Furthermore, we relied on visual inspection of spectral features as a means to identify fluctuations, but larger datasets might yield clues as to how an objective, automated method might be used to identify confounding state fluctuations.

Preserved physiological integrity characterizes cognitive motor dissociation

Our findings provide further insight into the underlying physiology that may give rise to a preserved capacity to reliably generate motor imagery to command in combination with an inability to communicate behaviourally. As previously proposed, both motor efferent loss and some significant impairment of corticothalamic function could lead to the manifestation of CMD (Fernández-Espejo *et al.*, 2015; Schiff, 2015). However, recent studies suggest a high degree of corticothalamic preservation in CMD, as indicated by globally preserved cerebral metabolism and the overall integrity of wakeful EEG background architecture in the time domain (Forgacs *et al.*, 2014; Stender *et al.*, 2014). Our results are consistent with these previous observations, as despite wide variation in CRS-R performance, responders to EEG command-following paradigms in our cohort overwhelmingly exhibited normal or mildly abnormal EEG background structure (Fig. 3). Thus, our data support an expectation for preserved brain function in the EEG command-following positive subject. CMD subjects can further be expected to show a mix of severely impaired motor outflow co-existing with widely preserved, functional corticothalamic systems capable of supporting goal-directed attention, working memory, and executive function (Owen *et al.*, 2006). Although CMD patients retain these substantial resources supporting their responsiveness to high-level mental imagery paradigms, distributed cerebral deafferentation leads to their functional sensitivity to ongoing variations in arousal regulation. As noted above, the evidence of low frequency power modulation in some patients reflects this co-existence of impaired cerebral networks and intact cognitive systems. Thus, the joint presence of low frequency power modulation and broad network integrity supporting mental imagery reflects a potential physiological correlate of these two co-existing substrates of preserved cognitive networks along with multi-focal deafferentation across the corticothalamic systems in CMD.

Although our study was not designed to assess frequency, our findings suggest a substantial prevalence of CMD among the general pool of DOC patients who lack a behavioural communication channel, with more than 40% of patient responders having a CRS-R communication subscale score of zero. One limitation to the generalizability of these results is that our patient studies are drawn from an anecdotal convenience sample and are thus likely biased through the enrolment of patients who remain alive and capable of participating in in-patient research studies long after severe brain injuries. Nonetheless, the high rate of positive EEG command-following seen in our convenience sample of subjects lacking communication channels is of particular concern. Independent of the limitations of our ability to estimate a prevalence of such persons, simply identifying such a large group, even in a convenience sample, is meaningful and warrants careful consideration. We note that our approach here is very conservative from a statistical point of view, requiring both consistency across runs and significance as determined by TGT with correction for FDR to account for multiple comparisons. Furthermore, we estimate a low rate of false positivity with our methods. Thus, our positive findings are supported by strong evidence.

An improvement of DOC assessment strategies is warranted, given the potentially high prevalence of CMD and the likely wide variability in characteristics of responses to tasks across the CMD population. Moreover, Edlow *et al.* (2017) have recently demonstrated that CMD may even be identified in the early stages of acute injury, further enlarging the need to develop tools and understanding of these types of electrophysiological signals. Standard evaluations of DOCs do not currently integrate multiple assessments and the use of quantitative methods to combine data obtained during similar arousal states, and thus, likely risk missing a significant portion of CMD patients. Moreover, an urgency exists for a large-scale screen of DOC patients to search for CMD, and the advantages and sensitivity of quantitative EEG methods when compared to other, non-behavioural measures make it an optimal tool for this effort.

The need for frequent and repeated testing to mitigate the potentially limiting impact of state fluctuations on accurate measurements (Fig. 6) (Wannez *et al.*, 2017), underscores the advantage of using electrophysiological measures to detect covert cognition in DOC patients over other neuroimaging measures (i.e. functional MRI) that have been used for the same purpose (Owen *et al.*, 2006; Monti *et al.*, 2010; Rodriguez Moreno *et al.*, 2010; Bardin *et al.*, 2011, 2012). In addition, the spatial variations associated with positive task performance suggest the use of several different paradigms, different techniques, and possible customization based on patient injury patterns. Notably, in our study, all patients with functional MRI evidence of command-following also demonstrated EEG evidence and seven patients demonstrated EEG evidence of command-following but not functional MRI evidence. Additionally, all five patients with contraindications to functional MRI demonstrated EEG evidence of command-following.

Our results are also consistent with many studies demonstrating that behavioural exams can be unreliable for detection of signs of consciousness in patients presenting with a clinical behavioural profile of the vegetative state or minimally conscious state (Schnakers *et al.*, 2009; Wannez *et al.*, 2017). In a recent study by Pignat *et al.* (2016), a non-standard assessment measure, the motor behavioural tool (MBT), was developed in an attempt to resolve more subtle, behavioural evidence of cognition not detected by the CRS-R. Although the MBT was shown to be effective in predicting recovery of consciousness in some instances, it may be subject to inaccuracy in the case of CMD patients with very severe structural injuries of the brainstem in combination with damage to corticothalamic oculomotor and motor control regions (Schiff, 2015). However, utilization of a combination of EEG command-following and enhanced behavioural assessment tools, such as the MBT, could lead to increased diagnostic accuracy of CMD patients and improved assessment of prevalence.

Implications for restoration of communication in cognitive motor dissociation

From the first description of a patient with a disorder of consciousness capable of performing mental tasks (Owen *et al.*, 2006), it has been recognized that high levels of preservation of many cognitive functions are manifest in such patients (Owen *et al.*, 2007). While it is thus clear that CMD patients are conscious and possess strong cognitive capacities, it is not certain that simply demonstrating command-following signals is sufficient to establish communication systems (Bardin *et al.*, 2011; Pokorny *et al.*, 2013). Nonetheless, once covert conscious awareness is detected in an individual, there exists an obligation to pursue attempts to restore communication, through either identifying an existing behavioural channel or incorporating the use of a BCI (Fins, 2015).

Of note, we tested multiple command-following paradigms in our subjects (four for most subjects reported here). As described above, each paradigm carries an independent risk for false positive outcomes and thus positive performance of one of the paradigms demonstrated here is not proposed as an evidentiary standard for the identification of either command-following capacity or CMD. Rather, the observation of very individualized response profiles suggests that refinements to future evaluation strategies should consider: (i) broad canvassing of possible reporting paradigms; (ii) narrowing of *a priori* hypotheses via identification of candidate spatial patterns and frequency bands for individual subjects; and (iii) well designed test-retest procedures controlling for the false positive rates of each test to account for multiple comparisons. Furthermore, any consistent task-related response as measured with the methods used here can be further tested to evaluate its use as a binary communication channel along with optimization for background state possibly improving the chance of success. Such a demonstration would provide definite evidence of the EEG signature reflecting network activity under high-level control of the executive language systems.

Our observation of the sensitivity of some patients' successful performance of mental imagery paradigms to arousal regulation raises challenges for BCI usage in disorders of consciousness (Fig. 6) (Lule *et al.*, 2013; Hauger *et al.*, 2015). Even in healthy volunteers, an inability to generate motor imagery has been shown to arise with only mild levels of pharmacologically-induced sedation, which associates with loss of supplementary motor area BOLD-functional MRI signal activation during motor imagery (Adapa *et al.*, 2012). BCI systems relying on neural signals associated with motor imagery may thus be subject to variable efficacy in DOC patients with impaired arousal regulation.

By contrast, our findings also reveal potential opportunities to improve BCI readiness in individuals identified with CMD. Most importantly, here we demonstrate tools that can both help to detect and, in the future, optimize potential use of command-following signals in communication systems designed for individual CMD subjects. The shift in characteristics of responses to a motor imagery task observed in one patient, before and after BCI usage (Fig. 7), suggests that global brain dynamics may change concurrently with recovery and BCI usage. During the second assessment, the characteristics of this patient's response were more similar to those of a healthy control subject, suggesting that characteristics of responses to these paradigms may provide insight into ongoing recovery. Based on our findings, we speculate that preservation of relatively normal EEG background and more preserved response pattern characteristics, similar to those seen in controls, may index or grade both recovery and likelihood of BCI readiness. In another study, functional, activity-dependent restructuring of brain networks associated with communication was observed in one patient recovering from severe brain injury (Thengone *et al.*, 2016). Thus, consistent engagement of a command-following channel may support a feedback process supporting recovery and leading to an enhanced likelihood of successfully harnessing a BCI. As Fins (2015) has argued, CMD patients with such a latent capacity have a right to appropriate recognition and associated rehabilitative efforts.

Acknowledgements

We thank Dr. Jonathan Victor for many helpful comments on earlier drafts of this manuscript and suggestions concerning EEG analysis. We also thank Dr. Tanya Nauvel and Jackie Gottshall for help with figure editing.

Funding

This study was supported by NIH HD51912, the James S. McDonnell Foundation, and the Jerold B. Katz Foundation.

Supplementary material

Supplementary material is available at *Brain* online.

References

Adapa R, Stamatakis E, Menon D. Assessment of hierarchy of motor function under sedation—using continuous arterial spin labeling. In: 18th Annual Meeting of the Organization for Human Brain Mapping. Beijing, China: Organization for Human Brain Mapping; 2012.

Allison JD, Meador KJ, Loring DW, Figueroa RE, Wright JC. Functional MRI cerebral activation and deactivation during finger movement. Neurology 2000; 54: 135–42.

Bai O, Lin P, Vorbach S, Floeter MK, Hattori N, Hallett M. A high performance sensorimotor beta rhythm-based brain-computer interface associated with human natural motor behavior. J Neural Eng 2008; 5: 24–35.

Bardin JC, Fins JJ, Katz DI, Hersh J, Heier LA, Tabelow K, et al. Dissociations between behavioural and functional magnetic resonance imaging-based evaluations of cognitive function after brain injury. Brain 2011; 134 (Pt 3): 769–82.

Bardin JC, Schiff ND, Voss HU. Pattern classification of volitional functional magnetic resonance imaging responses in patients with severe brain injury. Arch Neurol 2012; 69: 176–81.

Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Series B Methodol 1995; 57: 289–300.

Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. Ann Stat 2001; 29: 1165–88.

Bokil H, Andrews P, Kulkarni JE, Mehta S, Mitra PP. Chronux: a platform for analyzing neural signals. J Neurosci Methods 2010; 192: 146–51.

Boly M, Coleman MR, Davis MH, Hampshire A, Bor D, Moonen G, et al. When thoughts become action: an fMRI paradigm to study volitional brain activity in non-communicative brain injured patients. Neuroimage 2007; 36: 979–92.

Casali AG, Gosseries O, Rosanova M, Boly M, Sarasso S, Casali KR, et al. A theoretically based index of consciousness independent of sensory processing and behavior. Sci Transl Med 2013; 5: 198ra05.

Chaudhary U, Xia B, Silvoni S, Cohen LG, Birbaumer N. Brain-computer interface-based communication in the completely locked-in state. PLoS Biol 2017; 15: e1002593.

Cruse D, Chennu S, Chatelle C, Bekinschtein TA, Fernandez-Espejo D, Pickard JD, et al. Bedside detection of awareness in the vegetative state: a cohort study. Lancet 2011; 378: 2088–94.

Delorme A, Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods 2004; 134: 9–21.

Edlow BL, Chatelle C, Spencer CA, Chu CJ, Bodien YG, O'Connor KL, et al. Early detection of consciousness in patients with acute severe traumatic brain injury. Brain 2017; 140: 2399–414.

Fernández-Espejo D, Rossit S, Owen AM. A thalamocortical mechanism for the absence of overt motor behavior in covertly aware patients. JAMA Neurol 2015; 72: 1442–50.

Fins JJ. Rights come to mind: brain injury, ethics, and the struggle for consciousness. New York, NY: Cambridge University Press; 2015.

Fins JJ, Schiff ND. In search of hidden minds. Scientific American Mind, 2016. p. 44–51.

Fins JJ, Schiff ND, Foley KM. Late recovery from the minimally conscious state: ethical and policy implications. Neurology 2007; 68: 304–7.

Forgacs PB, Conte MM, Fridman EA, Voss HU, Victor JD, Schiff ND. Preservation of electroencephalographic organization in patients with impaired consciousness and imaging-based evidence of command-following. Ann Neurol 2014; 76: 869–79.

Fridman EA, Beattie BJ, Broft A, Laureys S, Schiff ND. Regional cerebral metabolic patterns demonstrate the role of anterior forebrain mesocircuit dysfunction in the severely injured brain. Proc Natl Acad Sci USA 2014; 111: 6473–8.

Friston KJ, Ashburner JT, Kiebel SJ, Nichols TE, Penny WD. Statistical parametric mapping: the analysis of functional brain images. Amsterdam: Elsevier; 2008.

Giacino JT, Fins JJ, Laureys S, Schiff ND. Disorders of consciousness after acquired brain injury: the state of the science. Nat Rev Neurol 2014; 10: 99–114.

Giacino JT, Kalmar K, Whyte J. The JFK coma recovery scale-revised: measurement characteristics and diagnostic utility. Arch Phys Med Rehabil 2004; 85: 2020–9.

Gibson RM, Chennu S, Fernandez-Espejo D, Naci L, Owen AM, Cruse D. Somatosensory attention identifies both overt and covert awareness in disorders of consciousness. Ann Neurol 2016; 80: 412–23.

Gibson RM, Fernandez-Espejo D, Gonzalez-Lara LE, Kwan BY, Lee DH, Owen AM, et al. Multiple tasks and neuroimaging modalities increase the likelihood of detecting covert awareness in patients with disorders of consciousness. Front Hum Neurosci 2014; 8: 950.

Gill-Thwaites H. Lotteries, loopholes and luck: misdiagnosis in the vegetative state patient. Brain Inj 2006; 20: 1321–8.

Goldfine AM, Bardin JC, Noirhomme Q, Fins JJ, Schiff ND, Victor JD. Reanalysis of "bedside detection of awareness in the vegetative state: a cohort study". Lancet 2013; 381: 289–91.

Goldfine AM, Victor JD, Conte MM, Bardin JC, Schiff ND. Determination of awareness in patients with severe brain injury using EEG power spectral analysis. Clin Neurophysiol 2011; 122: 2157–68.

Hauger SL, Schnakers C, Andersson S, Becker F, Moberget T, Giacino JT, et al. Neurophysiological indicators of residual cognitive capacity in the minimally conscious state. Behav Neurol 2015; 2015: 145913.

Hjorth B. An on-line transformation of EEG scalp potentials into orthogonal source derivations. Electroencephalogr Clin Neurophysiol 1975; 39: 526–30.

Hjorth B. Source derivation simplifies topographical EEG interpretation. Am J EEG Technol 1980; 20: 121–32.

Jasper HH. The ten-twenty electrode system of the International Federation. Electroencephalogr Clin Neurophysiol 1958; 10: 371–5.

Laureys S, Schiff ND. Coma and consciousness: paradigms (re)framed by neuroimaging. Neuroimage 2012; 61: 478–91.

Liu Y, Shen H, Zhou Z, Hu D. Sustained negative BOLD response in human fMRI finger tapping task. PLoS One 2011; 6: e23839.

Lule D, Noirhomme Q, Kleih SC, Chatelle C, Halder S, Demertzi A, et al. Probing command-following in patients with disorders of consciousness using a brain-computer interface. Clin Neurophysiol 2013; 124: 101–6.

Mazziotta J, Toga A, Evans A, Fox P, Lancaster J, Zilles K, et al. A probabilistic atlas and reference system for the human brain: International Consortium for Brain Mapping (ICBM). Philos Trans R Soc Lond B Biol Sci 2001; 356: 1293–322.

Monti MM, Vanhaudenhuyse A, Coleman MR, Boly M, Pickard JD, Tshibanda L, et al. Willful modulation of brain activity in disorders of consciousness. N Eng J Med 2010; 362: 579–89.

Owen AM, Coleman MR, Boly M, Davis MH, Laureys S, Jolles D, et al. Response to comments on "detecting awareness in the vegetative state". Science 2007; 315: 1221.

Owen AM, Coleman MR, Boly M, Davis MH, Laureys S, Pickard JD. Detecting awareness in the vegetative state. Science 2006; 313: 1402.

Percival DB, Walden AT. Spectral Analysis for Physical Applications. Cambridge: Cambridge University Press; 1993.

Pfurtscheller G, Lopes Da Silva FH. Event-related EEG/MEG synchronization and desynchronization: Basic principles. Clinical Neurophysiology 1999; 110: 1842–57.

Pfurtscheller G, Neuper C. Motor imagery activates primary sensorimotor area in humans. Neurosci Lett 1997; 239: 65–8.

Pignat JM, Mauron E, Johr J, Gilart de Keranflec'h C, Van De Ville D, Preti MG, et al. Outcome prediction of consciousness disorders in the acute stage based on a complementary motor behavioural tool. PLoS One 2016; 11: e0156882.

Pokorny C, Klobassa DS, Pichler G, Erlbeck H, Real RG, Kubler A, et al. The auditory P300-based single-switch brain-computer interface: paradigm transition from healthy subjects to minimally conscious patients. Artif Intell Med 2013; 59: 81–90.

Rodriguez Moreno D, Schiff ND, Giacino J, Kalmar K, Hirsch J. A network approach to assessing cognition in disorders of consciousness. Neurology 2010; 75: 1871–8.

Schiff ND. Recovery of consciousness after brain injury: a mesocircuit hypothesis. Trends Neurosci 2010; 33: 1–9.

Schiff ND. Cognitive motor dissociation following severe brain injuries. JAMA Neurol 2015; 72: 1413–15.

Schiff ND. Central thalamic deep brain stimulation to support anterior forebrain mesocircuit function in the severely injured brain. J Neural Transm 2016; 123: 797–806.

Schiff ND, Giacino JT, Kalmar K, Victor JD, Baker K, Gerber M, et al. Behavioural improvements with thalamic stimulation after severe traumatic brain injury. Nature 2007; 448: 600–3.

Schiff ND, Nauvel T, Victor JD. Large-scale brain dynamics in disorders of consciousness. Curr Opin Neurobiol 2014; 25: 7–14.

Schnakers C, Vanhaudenhuyse A, Giacino J, Ventura M, Boly M, Majerus S, et al. Diagnostic accuracy of the vegetative and minimally conscious state: clinical consensus versus standardized neurobehavioral assessment. BMC Neurol 2009; 9: 35.

Stender J, Gosseries O, Bruno MA, Charland-Verville V, Vanhaudenhuyse A, Demertzi A, et al. Diagnostic precision of PET imaging and functional MRI in disorders of consciousness: a clinical validation study. Lancet 2014; 384: 514–22.

Stender J, Mortensen KN, Thibaut A, Darkner S, Laureys S, Gjedde A, et al. The minimal energetic requirement of sustained awareness after brain injury. Curr Biol 2016; 26: 1494–9.

Thengone DJ, Voss HU, Fridman EA, Schiff ND. Local changes in network structure contribute to late communication recovery after severe brain injury. Sci Transl Med 2016; 8: 368re5.

Thickbroom GW, Mastaglia FL, Carroll WM, Davies HD. Source derivation: application to topographic mapping of visual evoked potentials. Electroencephalogr Clin Neurophysiol 1984; 59: 279–85.

Thomson DJ. Spectrum estimation and harmonic analysis. Proc IEEE 1982; 70: 1055–96.

Wannez S, Heine L, Thonnard M, Gosseries O, Laureys S. The repetition of behavioral assessments in diagnosis of disorders of consciousness. Ann Neurol 2017; 81: 883–9.

Williams ST, Conte MM, Goldfine AM, Noirhomme Q, Gosseries O, Thonnard M, et al. Common resting brain dynamics indicate a possible mechanism underlying zolpidem response in severe brain injury. Elife 2013; 2: e01157.