Supplementary Material

Early detection of consciousness in patients with acute severe traumatic brain injury

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Supplementary Methods

Neurobehavioural and outcome assessments

The Coma Recovery Scale-Revised (CRS-R) is comprised of 23 items hierarchically organized into six subscales assessing auditory, visual, motor and oromotor/verbal function in patients with disorders of consciousness (DoC) (Giacino *et al.*, 2004). It was used to characterize patients as being in a coma, vegetative state (VS), minimally conscious state without language preservation (MCS-; i.e., at least one of the following: visual fixation, visual pursuit, object localization, localization to noxious stimulation, object manipulation, automatic motor responses, or non-functional communication), minimally conscious state with language preservation (MCS+; i.e., at least one of the following: command-following, object recognition, or intelligible verbalisation (Bruno *et al.*, 2012; Giacino *et al.*, 2002; Schnakers *et al.*, 2015)), or emergence from the MCS (i.e. functional object use and/or functional communication). If a patient emerged from MCS, the diagnosis of post-traumatic confusional state (PTCS; (Stuss *et al.*, 1999)) was confirmed based on criteria derived from the Confusion Assessment Protocol (CAP) requiring the presence of three (if the patient was disoriented) or more than three (if the patient was oriented) of the following features: cognitive impairment, agitation, symptom fluctuation, nighttime sleep disturbance, decreased daytime arousal, or psychotic-type symptoms (Sherer *et al.*, 2005).

Functional outcome at six months post-injury was measured with the Extended Glasgow Outcome Scale (GOSE) (Wilson *et al.*, 1998). The GOSE is an eight-item self-reported outcome measure that is used extensively in TBI studies. Areas of assessment include independence (inside/outside the home), return to work and leisure activities, psychological function, and ongoing clinical symptoms.

Instructions given before and during the functional MRI

Prior to the MRI scan, all patients and healthy subjects were informed that language and music would be played via earphones during the MRI scan. The motor imagery task ("imagine squeezing your right hand") was also explained to all subjects, including patients whose behavioral examination suggested unconsciousness. During preparation for the MRI scan, the earphones were secured with tape to each subject's ears to prevent them from inadvertently falling out of the ears during the scan. Soft padding was placed between the head and the coil for subject comfort and to limit head motion.

Immediately before the MRI scan, patients and healthy subjects were instructed to lie still and close their eyes during the scan. Performing the fMRI paradigms with eyes closed ensured consistency in data acquisition across patients and healthy subjects, because patients in coma are unable to open their eyes. This eyes-closed approach is also consistent with methodology implemented in prior fMRI studies of patients with DoC (Bardin *et al.*, 2011).

During the MRI scan, pre-recorded verbal instructions were provided to the subjects via earphones before presentation of each paradigm. Transcripts of the verbal instructions are provided in Supplementary Table 1. All verbal instructions and auditory stimuli were prepared in Audacity (www.audacityteam.org) and administered via an iPod (Apple Inc.; Cupertino, CA) that was connected to the MRI scanner's sound system. The earphones simultaneously served as earplugs to attenuate scanner noise. Earphone volume was set at a comfortable level by healthy subjects or by study staff for patients who could not communicate.

Functional MRI data analysis

Functional MRI (fMRI) data acquisition, processing, and analysis methods are reported in accordance with guidelines published by the Organization for Human Brain Mapping Committee on Best Practices in Data Analysis and Sharing (COBIDAS) (Nichols *et al.*, 2016). Registration to T1 MEMPRAGE and standard space images (i.e. MNI152 T1 1mm) was carried out using FLIRT (Jenkinson *et al.*, 2002; Jenkinson and Smith, 2001), and the inverse of these transformations was used to register the regions of interest (ROIs) into native fMRI space. The following pre-statistics processing was applied: motion correction using MCFLIRT (Jenkinson *et al.*, 2002); non-brain removal using BET (Smith, 2002); spatial smoothing using a Gaussian kernel of FWHM 10 mm; grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor; high pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma=48.0s). Time-series statistical analysis was carried out using FILM with local autocorrelation correction (Woolrich *et al.*, 2001). Z (Gaussianised T/F) statistic images were thresholded using clusters determined by Z>3.1 and a (corrected) cluster significance threshold of p=0.05 (Worsley, 2001). The stringent statistical threshold for cluster significance (Z>3.1 versus Z>2.3) and the size of the Gaussian kernel (FWHM 10 mm versus FWHM 5 mm) were both selected based upon recent results indicating decreased false positive cluster activations using these parameters (Eklund *et al.*, 2016). To minimize the possibility of confounding due to motion, an additional motion correction procedure was used to supplement the standard MCFLIRT correction. We calculated rotational and translational motion parameters for each 4-dimensional fMRI dataset using the "fsl_motion_outliers" command, yielding a confound matrix that was included in the generalized linear model to completely remove the effects of these motion-degraded time points from the analysis.

Rationale for functional MRI paradigms and regions of interest

Because there are no prior stimulus-based fMRI studies focusing on patients with acute traumatic DoC, we selected *a priori* ROIs based upon fMRI studies of language, music and motor imagery in patients with chronic traumatic DoC and healthy human subjects. For the language task, we used ROIs for Heschl's gyrus and for the superior temporal gyrus (STG). Heschl's gyrus is proximal to Wernicke's area in the processing of auditory stimuli and its activation corresponds with primary (i.e., non-association) auditory cortex (Da Costa *et al.*, 2011). The STG, on the other hand, contains Wernicke's area, which is a central node in the network of association cortices that process the syntax, prosody, and semantic content of auditory stimuli (Price, 2012).

The Heschl's gyrus and STG ROIs were constructed as bilateral structures because 1) we did not exclude left-handed subjects for whom language may be represented bilaterally or predominantly in the right-hemisphere (Knecht *et al.*, 2000), and 2) bilateral STG activation is frequently observed in response to language (Coleman *et al.*, 2007; Fernandez-Espejo *et al.*, 2008) and music (Kovacs *et al.*, 2006; Okumura *et al.*, 2014) stimuli. Although Wernicke's area is believed to be localized to the posterior STG, we included the anterior STG in our STG ROI based upon recent evidence from human studies that word recognition in the anterior STG is dissociable from sentence recognition in the posterior STG (Mesulam *et al.*, 2015). Furthermore, anterior STG activation during language stimuli has been observed in healthy controls and patients with chronic DOC (Coleman *et al.*, 2009; Fernandez-Espejo *et al.*, 2008; Schiff *et al.*, 2005). We used the same Heschl's gyrus and STG ROIs for the language and music analyses because of the shared neural architecture involved in processing language and music stimuli (Koelsch, 2011; Patel, 2003). Moreover, the neuroanatomic convergence of linguistic and musical syntactical processing in bitemporal network hubs has been suggested by human fMRI, EEG, and magnetoencephalography data, as reviewed by (Patel, 2003). These findings suggest that both language and music stimuli can be used with fMRI and EEG to probe the brain's ability to process complex sound.

A right hand squeeze motor imagery task was selected instead of other motor imagery paradigms such as tennis or swimming (Bardin *et al.*, 2011; Boly *et al.*, 2007; Monti *et al.*, 2010; Owen *et al.*, 2006) because hand squeeze can be more readily compared to behavioral examinations performed in the ICU, during which patients are commonly asked to squeeze and release the examiner's hand. We repeated instructions to "keep squeezing" and "keep resting" throughout the task in case patients forgot the pre-task instructions. Notably, the instructions administered during the task have the same number of syllables and were repeated at consistent 6-second intervals to limit the possibility that the passive language stimulation might confound the measurement of brain responses specific to the active motor imagery task.

With regard to the ROI used for the motor imagery task, several experimental findings from fMRI and positron emission tomography motor imagery studies of healthy humans and patients with chronic DoC provided the rationale for creating a bilateral SMA/PMC ROI for a unilateral hand squeeze task: 1) hand/finger movement imagery is associated with both SMA and PMC activation (Grezes and Decety, 2001; Hanakawa *et al.*, 2008); 2) bilateral SMA and PMC activation is commonly observed in unilateral hand/finger imagery tasks (Grezes and Decety, 2001; Hanakawa *et al.*, 2008); and 3) although the dorsal component of the PMC (i.e. PMD) is believed to be the most neuroanatomically specific region activated during preparation/intention of hand movement (Pilgramm *et al.*, 2016), activation typically extends beyond PMD to the ventral component of the PMC (PMV) (Nedelko *et al.*, 2012). Together, these data suggest that a spatially inclusive SMA/PMC ROI may be necessary to detect willful brain responses during a hand squeeze motor imagery task. We did not include the posterior parietal cortex in our motor imagery ROI, because parietal activation is less robust in hand and finger motor imagery paradigms that lack spatial complexity (Grafton *et al.*, 1992; Kuhtz-Buschbeck *et al.*, 2003), as compared to more complex motor tasks such as swimming or playing tennis (Bardin *et al.*, 2011; Boly *et al.*, 2007; Monti *et al.*, 2010; Owen *et al.*, 2006).

We created the SMA/PMC ROI by combining the SMA ROI from the Harvard-Oxford Cortical Atlas (Makris *et al.*, 2006) with the right and left PMC ROIs from the Juelich Histological Atlas (Eickhoff *et al.*, 2005), as distributed in FSLView v3.1.8 (Smith *et al.*, 2004). The atlas-based regions and thresholding parameters used to generate the final ROIs for each paradigm are provided in Supplementary Table 2. Of note, the SMA/PMC ROI generated here includes aspects of the precentral gyrus that may approach or overlap primary motor cortex (BA4), as seen in the bottom right panel of Figure 1. We did not attempt to distinguish between fMRI responses within PMC (BA6) and primary motor cortex (BA4). We implementated all ROI-based analyses during the FEATQuery processing step (after the second-level analysis), rather than using a pre-threshold masking ROI during the FEAT first-level analysis. This analytic approach made our multiple-comparison correction more stringent, thus reducing the likelihood of false-positive responses within each ROI.

Instructions given before and during the EEG

As with the stimulus-based fMRI experiments, a study investigator instructed all subjects to lie still and keep their eyes closed prior to the EEG. Subjects were again informed that language and music stimuli would be played, and instructions regarding the motor imagery task were repeated. Each patient and control subject was lying supine on a hospital bed (i.e. in the ICU or EEG laboratory), with the head of bed elevated at 30 degrees. Speakers were placed at the level of the subject's ears at a volume that was comfortable for the subject, or in the case of patients who could not reliably communicate, at a volume that was comfortable for the investigator. Ambient noise was minimized by closing the door to the rooms in which data were acquired for patients and healthy subjects, but ventilator and hemodynamic alarms remained audible for patients in accordance with standard ICU procedures. As with the fMRI motor imagery paradigm, the EEG motor imagery paradigm included instructions that were repeated at 6-second intervals throughout the task (e.g., "keep squeezing", "keep resting").

EEG classification using power spectral density

Following artifact rejection, we estimated the power spectral density of the voltage activity recorded at each of 19 electrodes using window lengths of T = 1 s, time-bandwidth product TW = 1, which precluded multitaper estimates but allowed a 1 Hz frequency resolution. All spectrograms were computed with the Chronux toolbox (Mitra and Bokil, 2008). Absolute power estimates were averaged within four frequency bands (delta [1-3 Hz], theta [4-7 Hz], alpha [8-13 Hz], beta [14-30 Hz]), resulting in a matrix (76 features [4 bands x 19 electrodes] by 576 seconds [24 blocks x 24 seconds]) that was used for classifier analysis for the language and music paradigms. The motor paradigm matrix had a shorter duration (76 features by 388 seconds) due to the removal of data collected during repeated instructions for that paradigm. Any segments where data had been removed following artifact rejection were padded to preserve indexing between trials. To minimize a potential bleeding effect, whereby brain activity occurring during a language stimulus may persist into a rest block (Wu *et al.*, 2013), we excluded data collected during the first second after a repeated instruction ("keep squeezing", "keep resting") during the motor imagery paradigm.

Considerations regarding medications for sedation, anxiolysis and analgesia

Due to differences in body mass index, renal metabolism, and hepatic metabolism, the impact of a particular medication regimen on an individual patient's brain function is difficult to quantify. Of particular note, multiple patients in our sample had a history of polysubstance abuse and demonstrated high tolerance to opioid and GABA-ergic medications (e.g., propofol and lorazepam). Consequently, we observed qualitatively (i.e., based on comparison of behavioural and motor responses pre- versus post-medication administration) that patients who received high doses of sedatives, anxiolytics, or analgesics were not necessarily as pharmacologically sedated as patients who received far lower doses of the same medications.

Also of note, for the 16 healthy subjects, electrophysiological evidence of Stage I sleep or Stage II sleep was detected during the majority of a given paradigm for language in 4 subjects, music in 2 subjects, and motor imagery in 1 subject. Nevertheless, we include these healthy subjects in our analyses to ensure consistency in the comparison between control and patient data, since reliable electrophysiological markers of sleep in brain-injured patients have not been defined (Foreman *et al.*, 2015).

Considerations regarding epileptiform discharges during EEG acquisition

We reviewed the EEG recordings for each patient for epileptiform activity and recorded the presence/absence of epileptiform discharges as well as their abundance (if sporadic) or frequency (if periodic) (see Supplementary Table 5). Grading of abundance and frequency was performed in accordance with American Clinical Neurophysiology Society criteria (Hirsch *et al.*, 2013). We found that three patients (P7, P8, and P13) had epileptiform discharges, and these patients had variable rates of positive EEG responses to the language (2/3), music (1/3) and motor imagery (0/3) stimuli. Of the 11 patients who did not have epileptiform activity, there were also variable rates of EEG responses to the language (7/11), music (7/11) and motor imagery (3/11) stimuli. Thus, there is no readily apparent association, in our limited sample, between epileptiform activity and the rate of EEG responses to language, music, or motor imagery stimuli. Given that there were only 3 patients with epileptiform activity, we do not have sufficient sample size to perform post-hoc statistical tests for a potential association between epileptiform activity and EEG responses.

Supplementary References

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: 769-82.

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.

Bruno MA, Majerus S, Boly M, Vanhaudenhuyse A, Schnakers C, Gosseries O, et al. Functional neuroanatomy underlying the clinical subcategorization of minimally conscious state patients. Journal of neurology 2012; 259: 1087-98.

Coleman MR, Davis MH, Rodd JM, Robson T, Ali A, Owen AM, et al. Towards the routine use of brain imaging to aid the clinical diagnosis of disorders of consciousness. Brain 2009; 132: 2541-52.

Coleman MR, Rodd JM, Davis MH, Johnsrude IS, Menon DK, Pickard JD, et al. Do vegetative patients retain aspects of language comprehension? Evidence from fMRI. Brain 2007; 130: 2494-507.

Da Costa S, van der Zwaag W, Marques JP, Frackowiak RS, Clarke S, Saenz M. Human primary auditory cortex follows the shape of Heschl's gyrus. J Neurosci 2011; 31: 14067-75.

Eickhoff SB, Stephan KE, Mohlberg H, Grefkes C, Fink GR, Amunts K, et al. A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. Neuroimage 2005; 25: 1325-35.

Eklund A, Nichols TE, Knutsson H. Cluster failure: Why fMRI inferences for spatial extent have inflated false-positive rates. Proc Natl Acad Sci U S A 2016; 113: 7900-5.

Fernandez-Espejo D, Junque C, Vendrell P, Bernabeu M, Roig T, Bargallo N, et al. Cerebral response to speech in vegetative and minimally conscious states after traumatic brain injury. Brain Inj 2008; 22: 882-90.

Foreman B, Westwood AJ, Claassen J, Bazil CW. Sleep in the neurological intensive care unit: feasibility of quantifying sleep after melatonin supplementation with environmental light and noise reduction. Journal of clinical neurophysiology : official publication of the American Electroencephalographic Society 2015; 32: 66-74.

Giacino JT, Ashwal S, Childs N, Cranford R, Jennett B, Katz DI, et al. The minimally conscious state: definition and diagnostic criteria. Neurology 2002; 58: 349-53.

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.

Grafton ST, Mazziotta JC, Woods RP, Phelps ME. Human functional anatomy of visually guided finger movements. Brain 1992; 115 (Pt 2): 565-87.

Grezes J, Decety J. Functional anatomy of execution, mental simulation, observation, and verb generation of actions: a meta-analysis. Hum Brain Mapp 2001; 12: 1-19.

Hanakawa T, Dimyan MA, Hallett M. Motor planning, imagery, and execution in the distributed motor network: a time-course study with functional MRI. Cereb Cortex 2008; 18: 2775-88.

Hirsch LJ, LaRoche SM, Gaspard N, Gerard E, Svoronos A, Herman ST, et al. American Clinical Neurophysiology Society's Standardized Critical Care EEG Terminology: 2012 version. Journal of clinical neurophysiology : official publication of the American Electroencephalographic Society 2013; 30: 1-27.
Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage 2002; 17: 825-41.
Jenkinson M, Smith S. A global optimisation method for robust affine registration of brain images. Medical image analysis 2001; 5: 143-56.
Knecht S, Drager B, Deppe M, Bobe L, Lohmann H, Floel A, et al. Handedness and hemispheric language dominance in healthy humans. Brain 2000; 123 Pt 12: 2512-8.
Koelsch S. Toward a neural basis of music perception - a review and updated model. Frontiers in psychology 2011; 2: 110.
Kovacs S, Peeters R, Smits M, De Ridder D, Van Hecke P, Sunaert S. Activation of cortical and subcortical auditory structures at 3 T by means of a functional magnetic resonance imaging paradigm suitable for clinical use. Investigative radiology 2006; 41: 87-96.
Kuhtz-Buschbeck JP, Mahnkopf C, Holzknecht C, Siebner H, Ulmer S, Jansen O. Effector-independent representations of simple and complex imagined finger movements: a combined fMRI and TMS study. Eur J Neurosci 2003; 18: 3375-87.
Makris N, Goldstein JM, Kennedy D, Hodge SM, Caviness VS, Faraone SV, et al. Decreased volume of left and total anterior insular lobule in schizophrenia. Schizophrenia research 2006; 83: 155-71.
Mesulam MM, Thompson CK, Weintraub S, Rogalski EJ. The Wernicke conundrum and the anatomy of language comprehension in primary progressive aphasia. Brain 2015; 138: 2423-37.
Mitra P, Bokil H. Observed Brain Dynamics. New York, NY: Oxford University Press; 2008.
Monti MM, Vanhaudenhuyse A, Coleman MR, Boly M, Pickard JD, Tshibanda L, et al. Willful modulation of brain activity in disorders of consciousness. N Engl J Med 2010; 362: 579-89.
Nedelko V, Hassa T, Hamzei F, Schoenfeld MA, Dettmers C. Action imagery combined with action observation activates more corticomotor regions than action observation alone. Journal of neurologic physical therapy : JNPT 2012; 36: 182-8.
Nichols TE, Das S, Eickhoff SB, Evans AC, Glatard T, Hanke M, et al. Best Practices in Data Analysis and Sharing in Neuroimaging using MRI. bioRxiv doi: 101101/054262 2016.
Okumura Y, Asano Y, Takenaka S, Fukuyama S, Yonezawa S, Kasuya Y, et al. Brain activation by music in patients in a vegetative or minimally conscious state following diffuse brain injury. Brain Inj 2014; 28: 944-50.
Owen AM, Coleman MR, Boly M, Davis MH, Laureys S, Pickard JD. Detecting awareness in the vegetative state. Science 2006; 313: 1402.
Patel AD. Language, music, syntax and the brain. Nat Neurosci 2003; 6: 674-81.

Pilgramm S, de Haas B, Helm F, Zentgraf K, Stark R, Munzert J, et al. Motor imagery of hand actions: Decoding the content of motor imagery from brain activity in frontal and parietal motor areas. Hum Brain Mapp 2016; 37: 81-93.
Price CJ. A review and synthesis of the first 20 years of PET and fMRI studies of heard speech, spoken language and reading. Neuroimage 2012; 62: 816-47.
Schiff ND, Rodriguez-Moreno D, Kamal A, Kim KH, Giacino JT, Plum F, et al. fMRI reveals large-scale network activation in minimally conscious patients. Neurology 2005; 64: 514-23.
Schnakers C, Edlow BL, Chatelle C, Giacino J. Minimally conscious state. In: Laureys S, Gosseries O, Tononi G, editors. The Neurology of Consciousness. 2nd ed. San Diego, CA: Academic Press; 2015.
Sherer M, Nakase-Thompson R, Yablon SA, Gontkovsky ST. Multidimensional assessment of acute confusion after traumatic brain injury. Arch Phys Med Rehabil 2005; 86: 896-904.
Smith SM. Fast robust automated brain extraction. Hum Brain Mapp 2002; 17: 143-55.
Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, et al. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage 2004; 23 Suppl 1: S208-19.
Stuss DT, Binns MA, Carruth FG, Levine B, Brandys CE, Moulton RJ, et al. The acute period of recovery from traumatic brain injury: posttraumatic amnesia or posttraumatic confusional state? J Neurosurg 1999; 90: 635-43.
Wilson JT, Pettigrew LE, Teasdale GM. Structured interviews for the Glasgow Outcome Scale and the extended Glasgow Outcome Scale: guidelines for their use. J Neurotrauma 1998; 15: 573-85.
Woolrich MW, Ripley BD, Brady M, Smith SM. Temporal autocorrelation in univariate linear modeling of FMRI data. Neuroimage 2001; 14: 1370-86.
Worsley K. Statistical analysis of activation images. In: Jezzard P, Matthews PM, Smith SM, editors. Functional MRI: An introduction to methods. Oxford: Oxford University Press; 2001.
Wu J, Zhang J, Ding X, Li R, Zhou C. The effects of music on brain functional networks: a network analysis. Neuroscience 2013; 250: 49-59.

Supplementary Tables

Supplementary Table 1: Instructions given prior to and during functional MRI

	Language	Music	Motor Imagery*
Instructions Prior to Stimulus	When the MRI starts, close your eyes and relax. Part of the time you will hear spoken words and part of the time you will hear silence. Keep your eyes closed the whole time.	When the MRI starts, close your eyes and relax. Part of the time you will hear music and part of the time you will hear silence. Keep your eyes closed the whole time.	When the MRI starts, close your eyes and relax. After about a minute, you will hear an instruction to squeeze your right hand. When you hear this, try to imagine that you are squeezing your right hand into a fist and then relaxing it. Concentrate on the way your muscles would feel if you were really performing this movement. Try to do this every time you hear the command to squeeze your right hand. Then, you will hear an instruction to stop. When you hear “stop”, stop squeezing your hand, relax, and keep your eyes closed.
Instructions During Stimulus	Not applicable	Not applicable	At the beginning of the task, the subject hears “Now squeeze your right hand.” Then every 6 seconds the subject hears, “keep squeezing.” At the beginning of rest, the subject hears, “Now stop and rest.” Then every 6 sec, the subject hears “keep resting.”

Supplementary Table 1 Legend: * The instructions for the motor imagery task were adapted from Cruse et al. Lancet 2011;378:2088-94.

Supplementary Table 2: Regions of interest for stimulus-based functional MRI analysis

ROI	Atlas	Atlas-Based Regions Included	Thresholding
Heschl's Gyrus	Harvard-Oxford Cortical Structural Atlas (Makris *et al.*, 2006)	Heschl's Gyrus (included H1 and H2)	5%
Superior Temporal Gyrus	Harvard-Oxford Cortical Structural Atlas (Makris *et al.*, 2006)	Superior Temporal Gyrus, anterior division + Superior Temporal Gyrus, posterior division	5% for both component ROIs
Supplementary Motor Area/ Premotor Cortex	Harvard-Oxford Cortical Structural Atlas (Makris *et al.*, 2006) and Juelich Histological Atlas (Eickhoff *et al.*, 2005)	Juxtapositional Lobule Cortex (formerly Supplementary Motor Cortex) + GM Premotor cortex BA6 L + GM Premotor Cortex BA6 R	5% for Juxtapositional Lobule Cortex ROI and 25% for GM Premotor Cortex ROIs

Supplementary Table 2 Legend: Each region of interest (ROI) was constructed using atlas-based neuroanatomic regions distributed with FSLView v3.1.8 (Smith *et al.*, 2004). For the language and the music fMRI paradigms, we used the bilateral Heschl's gyrus and superior temporal gyrus (anterior and posterior) ROIs distributed by the Harvard-Oxford Cortical Structural Atlas, each of which was thresholded at 5% and binarized using FSLMaths. For the motor imagery fMRI paradigm, we combined regions distributed by Harvard-Oxford Cortical Structural Atlas (Makris *et al.*, 2006) and the Juelich Histological Atlas (Eickhoff *et al.*, 2005). The Brodmann area (BA) 6 ROI of the Juelich Histological Atlas is comprised of the supplemental motor area (SMA), pre-SMA, and the four zones of the premotor cortex: premotor dorsal rostral (PMDr), premotor dorsal caudal (PMDc), premotor ventral rostral (PMVr), and premotor ventral caudal (PMVc). Of note, the pre-SMA is a common site of activation in fMRI motor imagery paradigms (Boly *et al.*, 2007). We added the Juxtapositional Lobule Cortex (i.e. SMA) ROI from the Harvard-Oxford Cortical Atlas because this ROI encompasses more SMA voxels along its inferior border that the Juelich BA6 ROI, and because it has been used previously in motor imagery fMRI studies of patients with DOC (Bardin *et al.*, 2011; Monti *et al.*, 2010). The BA6 ROIs from the Juelich Histological Atlas were thresholded at 25%, and the Juxtapositional Lobule Cortex ROI from the Harvard-Oxford Cortical Structural Atlas was thresholded at 5% prior to concatenation and binarization using FSLmaths.

Supplementary Table 3: Healthy subject responses to stimulus-based functional MRI and EEG

ID	Age	Sex	Language fMRI (% activated voxels)		Music fMRI (% activated voxels)		Motor Imagery fMRI (% activated voxels)	Language Forwards > Backwards fMRI (% activated voxels)	Language EEG P Value	Music EEG P Value	Motor Imagery EEG P Value
			Heschl	STG	Heschl	STG	SMA/PMC	STG			
C1	27	M	79.0%	61.8%	56.8%	50.6%	0.0%	0.0%	0.002	< 0.001	< 0.001
C2	21	M	68.4%	77.6%	82.3%	71.6%	46.6%	10.9%	< 0.001	0.014	< 0.001
C3	27	M	0.8%	4.5%	65.8%	39.1%	0.4%	0.0%	< 0.001	0.076	0.044
C4	21	M	75.7%	96.6%	58.2%	33.3%	19.4%	9.9%	< 0.001	< 0.001	0.114
C5	19	F	32.9%	49.7%	71.9%	53.1%	0.7%	0.0%	0.066	0.010	< 0.001
C6	19	M	72.8%	73.4%	87.6%	18.5%	1.4%	0.0%	< 0.001	< 0.001	0.002
C7	34	M	44.6%	56.0%	38.4%	8.2%	2.4%	0.1%	< 0.001	< 0.001	< 0.001
C8	28	F	55.2%	83.0%	85.0%	54.2%	2.8%	5.9%	0.002	< 0.001	0.002
C9	45	M	71.6%	91.9%	48.9%	36.1%	2.2%	13.7%	< 0.001	0.376	0.020
C10	33	M	59.6%	46.1%	83.4%	46.9%	8.3%	0.0%	0.002	< 0.001	0.016
C11	32	M	47.7%	76.7%	78.2%	62.6%	0.0%	6.3%	0.128	0.012	0.008
C12	24	M	4.5%	6.3%	18.8%	8.6%	0.0%	0.0%	< 0.001	0.010	0.112
C13	22	F	47.9%	47.2%	0.0%	0.0%	0.0%	0.0%	< 0.001	0.004	< 0.001
C14	27	F	59.6%	83.7%	52.6%	19.5%	4.3%	11.3%	< 0.001	0.020	0.058
C15	18	M	25.0%	79.6%	6.8%	0.04%	0.0%	7.5%	< 0.001	< 0.001	0.106
C16	51	M	62.9%	71.0%	58.5%	27.71%	4.3%	2.3%	< 0.001	< 0.001	< 0.001
Group Median			57.4%	72.2%	58.5%	36.1%	1.8%	1.2%	N/A	N/A	N/A
Group Range [2.5% to 97.5%]			2.2% to 77.8%	5.2% to 94.8%	2.6% to 86.6%	0.02% to 68.3%	0.0% to 36.4%	0.0% – 12.8%	N/A	N/A	N/A

Supplementary Table 3 Legend: Abbreviations: F = female; fMRI = functional magnetic resonance imaging; Heschl = Heschl's gyrus; M = male; SMA/PMC = supplementary motor area/premotor cortex; STG = superior temporal gyrus.

Supplementary Table 4: Sedative, anxiolytic and analgesic medications administered before and during fMRI and EEG

ID	LoC at fMRI/EEG	Medications Administered Before and During fMRI	Medications Administered Before and During EEG
P1	PTCS/ PTCS	Before MRI: diazepam 2.5 mg PGT During MRI: morphine 6 mg IV	Before EEG: None During EEG: None
P2	MCS-/ MCS-	Before MRI: None During MRI: None	Before EEG: morphine 2 mg IV During EEG: None
P3	Coma/ Coma	Before MRI: propofol 300 mg/hr IV gtt During MRI: propofol 300 mg/hr IV gtt	Before EEG: propofol 100 mg/hr IV gtt During EEG: propofol 100 mg/hr IV gtt + propofol 10 mg IV bolus before motor imagery
P4	PTCS/ PTCS	Before MRI: None During MRI: None	Before EEG: None During EEG: None
P5	VS/ VS	Before MRI: ativan 1 mg IV During MRI: None	Before EEG: oxycodone 5 mg PGT During EEG: quetiapine 25 mg PGT
P6	VS/ MCS+	Before MRI: hydromorphone 0.5 mg IV During MRI: hydromorphone 0.5 mg IV	Before EEG: hydromorphone 0.5 mg IV During EEG: None
P7	MCS+/ MCS+	Before MRI: quetiapine 12.5 mg PGT During MRI: None	Before EEG: quetiapine 12.5 mg PGT During EEG: None
P8	PTCS/ PTCS	Before MRI: propofol 300 mg/hr IV gtt During MRI: propofol 400 mg/hr IV gtt + hydromorphone 0.5 mg IV	Before EEG: propofol 300 mg/hr IV gtt During EEG: propofol 300 mg/hr IV gtt + hydromorphone 0.5 mg IV
P9	MCS+/ MCS+	Before MRI: propofol 200 mg/hr IV gtt During MRI: propofol 200 mg/hr IV gtt	Before EEG: None During EEG: None
P10	MCS-/ MCS-	Before MRI: None During MRI: None	Before EEG: None During EEG: None
P11	PTCS/ PTCS	Before MRI: lorazepam 1 mg IV During MRI: haloperidol 5 mg IV	Before EEG: lorazepam 1 mg IV + haloperidol 5 mg IV During EEG: None
P12	Coma/ Coma	Before MRI: propofol 50 mg/hr IV gtt During MRI: propofol 50 mg/hr IV gtt	Before EEG: midazolam 2 mg IV During EEG: None
P13	MCS+/ PTCS	Before MRI: propofol 300 mg/hr IV gtt During MRI: propofol 300 mg/hr IV gtt	Before EEG: None During EEG: None
P14	VS/	Before MRI: fentanyl 50 mcg IV bolus + propofol 20 mg IV bolus	Before EEG: N/A

	N/A	During MRI: propofol 25 mg/hr IV gtt	During EEG: N/A
P15	MCS-/ MCS+	Before MRI: propofol 300 mg/hr IV gtt + fentanyl 50 mcg IV bolus During MRI: propofol 200 mg/hr IV gtt	Before EEG: None During EEG: None
P16	MCS+/ PTCS	Before MRI: propofol 300 mg/hr IV gtt + fentanyl 50 mcg IV bolus During MRI: propofol 300 mg/hr IV gtt	Before EEG: propofol 300 mg/hr IV gtt + fentanyl 50 mcg IV bolus During EEG: propofol 300 mg/hr IV gtt

Supplementary Table 4 Legend: Level of Consciousness (LoC) was assessed via behavioural evaluation with the Coma Recovery Scale-Revised as coma, vegetative state (VS), minimally conscious state without language function (MCS-), minimally conscious state with language function (MCS+), or post-traumatic confusional state (PTCS; emerged from MCS but disoriented). Abbreviations: fMRI = functional MRI; gtt = continuous infusion; IV = intravenous; LoC = level of consciousness; PGT = administered via percutaneous gastric tube.

Supplementary Table 5: Anti-seizure medications and epileptiform activity

ID	Anti-seizure Medication(s)	Epileptiform Discharges?	Abundance (if sporadic) or Frequency (if periodic)
P1	none	No	N/A
P2	levetiracetam 500 mg IV BID	No	N/A
P3	levetiracetam 500 mg PNGT BID	No	N/A
P4	none	No	N/A
P5	none	No	N/A
P6	levetiracetam 500 mg POGT BID	No	N/A
P7	none	Yes	occasional
P8	none	Yes	occasional
P9	none	No	N/A
P10	none	No	N/A
P11	none	No	N/A
P12	none	N/A[a]	N/A[a]
P13	levetiracetam 500 mg IV BID, valproic acid 250 mg PNGT qAM, 750 mg PNGT qHS	Yes	occasional
P14	N/A[b]	N/A[b]	N/A[b]
P15	levetiracetam 750 mg PNGT BID	No	N/A
P16	levetiracetam 500 mg PNGT BID	No	N/A

Supplementary Table 5 Legend: Epileptiform activity observed during the research EEG is reported. Specifically, we report sporadic and periodic discharges and their abundance or frequency, respectively. Presence of epileptiform activity was determined based on visual inspection of the EEG recordings. Anti-seizure medications administered within the prior 24 hours are reported. Grading of abundance and frequency was performed in accordance with American Clinical Neurophysiology Society criteria (Hirsch *et al.*, 2013). The term "occasional" refers to a frequency range of 1 per hour to 1 per minute. [a] EEG data unusable due to myogenic artifact. [b] Patient died due to withholding of life-sustaining treatment. Abbreviations: BID = twice per day; IV = intravenous; PGT = administered via percutaneous gastric tube; N/A = not applicable; PNGT = administered via nasogastric tube; POGT = administered via orogastric tube; qAM = in the morning; qHS = at bedtime.

Supplementary Table 6: Patient responses to stimulus-based functional MRI and EEG

ID	LoC at fMRI/EEG	Language fMRI (% activated voxels)		Music fMRI (% activated voxels)		Motor Imagery fMRI (% activated voxels)	Language Forwards > Backwards fMRI (% activated voxels)	Language EEG P Value	Music EEG P Value	Motor Imagery EEG P Value	GOSE at F/U	CMD or HMD
		Heschl	STG	Heschl	STG	SMA/PMC	STG					
P1	PTCS/PTCS	29.1%	47.2%	22.4%	13.7%	0.1%	0.5%	0.014	< 0.001	0.014	3	N/A
P2	MCS-/MCS-	43.0%	31.3%	30.0%	7.9%	0.0%	1.4%	0.012	< 0.001	0.262	7	HMD
P3	Coma/Coma	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.430	0.086	0.186	7	No
P4	PTCS/PTCS	0.0%	0.0%	N/A[a]	N/A[a]	N/A[a]	15.4%	< 0.001	N/A[b]	N/A[b]	7	N/A
P5	VS/VS	0.0%	0.0%	0.0%	0.0%	0.5%	0.0%	0.068	0.710	0.058	3	CMD
P6	VS/MCS+	31.3%	26.6%	25.2%	9.4%	0.3%	3.0%	0.006	0.008	0.116	3	CMD
P7	MCS+/MCS+	24.9%	17.4%	58.2%	29.7%	0.0%	0.0%	0.314	0.120	0.078	5	N/A
P8	PTCS/PTCS	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.014	0.362	0.064	3	N/A
P9	MCS+/MCS+	17.9%	11.0%	31.0%	16.7%	0.0%	0.4%	< 0.001	< 0.001	< 0.001	4	N/A
P10	MCS-/MCS-	21.5%	28.8%	0.0%	0.0%	2.0%	0.0%	0.522	0.024	0.300	5	CMD
P11	PTCS/PTCS	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.228	0.010	0.036	7	N/A
P12	Coma/Coma	0.0%	0.0%	0.0%	0.0%	0.0%	0.1%	N/A[c]	N/A[c]	N/A[c]	1	No
P13	MCS+/PTCS	43.3%	8.6%	12.5%	0.0%	2.2%	0.0%	< 0.001	0.004	0.626	7	N/A
P14	VS/N/A[d]	0.0%	2.7%[e]	3.0%	9.0%	0.9%	0.0%	N/A[d]	N/A[d]	N/A[d]	1	CMD
P15	MCS-/MCS+	40.2%	50.3%	0.0%	0.0%	0.0%	0.0%	< 0.001	< 0.001	0.352	5	HMD
P16	MCS+/PTCS	37.0%	37.0%	11.9%	4.8%	7.5%	15.1%	< 0.001	0.490	0.630	5	N/A

Supplementary Table 6 Legend: Level of Consciousness (LoC) is assessed via behavioural evaluation with the Coma Recovery Scale-Revised (CRS-R) as coma, vegetative state (VS), minimally conscious state without language function (MCS-), minimally conscious state with language function (MCS+), or post-traumatic confusional state (PTCS; emerged from MCS but disoriented). Cognitive motor dissociation (CMD) is defined by functional MRI (fMRI) or EEG evidence of command-following on the motor imagery task despite behavioural absence of language function. Higher-order cortex motor dissociation (HMD) is defined as fMRI *and* EEG evidence of cortical responses to passive language or music stimuli despite behavioural absence of language. [a] Unable to complete fMRI due to severe agitation. [b] EEG data unusable due to motion artifact. [c] EEG data unusable due to myogenic artifact. [d] Patient died due to withholding of life-sustaining treatment. [e] indicates that P14 had fMRI activation within the superior temporal gyrus (STG) during language, but because there was no concurrent activation in primary auditory cortex (i.e. Heschl's gyrus), P14 did not meet our *a priori* criteria for a positive response to language. Abbreviations: F = female; F/U = follow up; fMRI = functional MRI; GOSE = Glasgow Outcome Scale-Extended; Heschl = Heschl's gyrus; M = male; N/A = not applicable; Ped = pedestrian; SMA/PMC = supplementary motor area/premotor cortex.

Supplementary Figures

Early detection of consciousness in patients with acute severe traumatic brain injury

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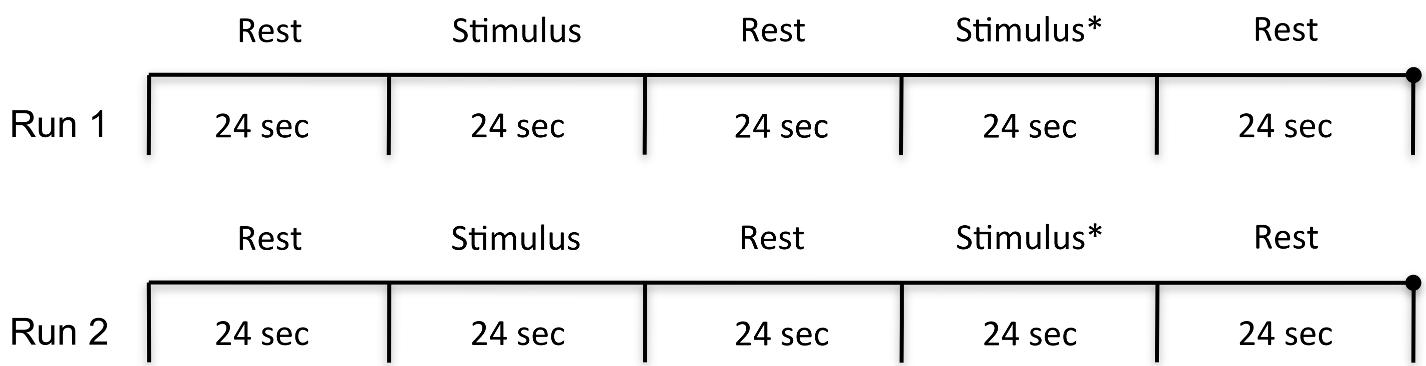
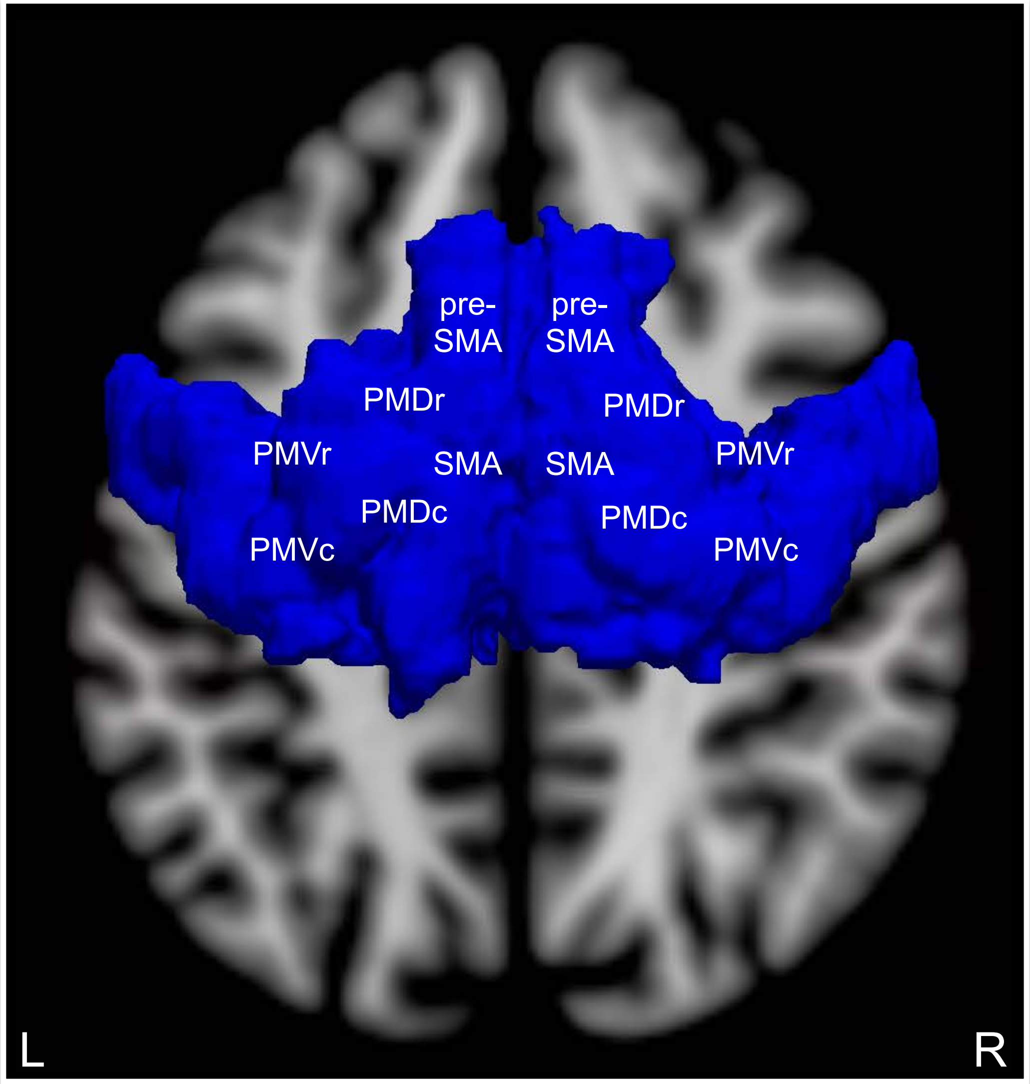
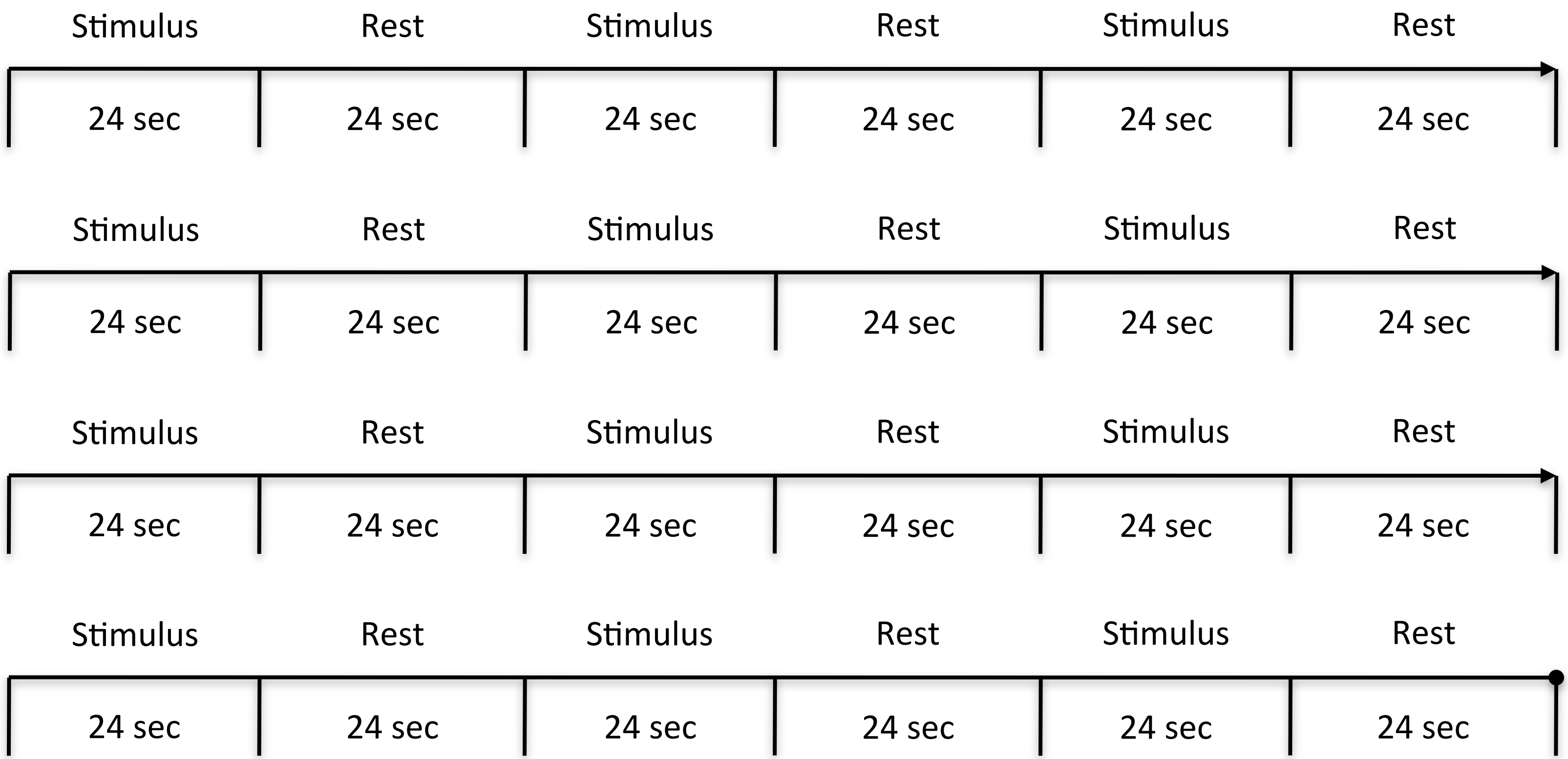


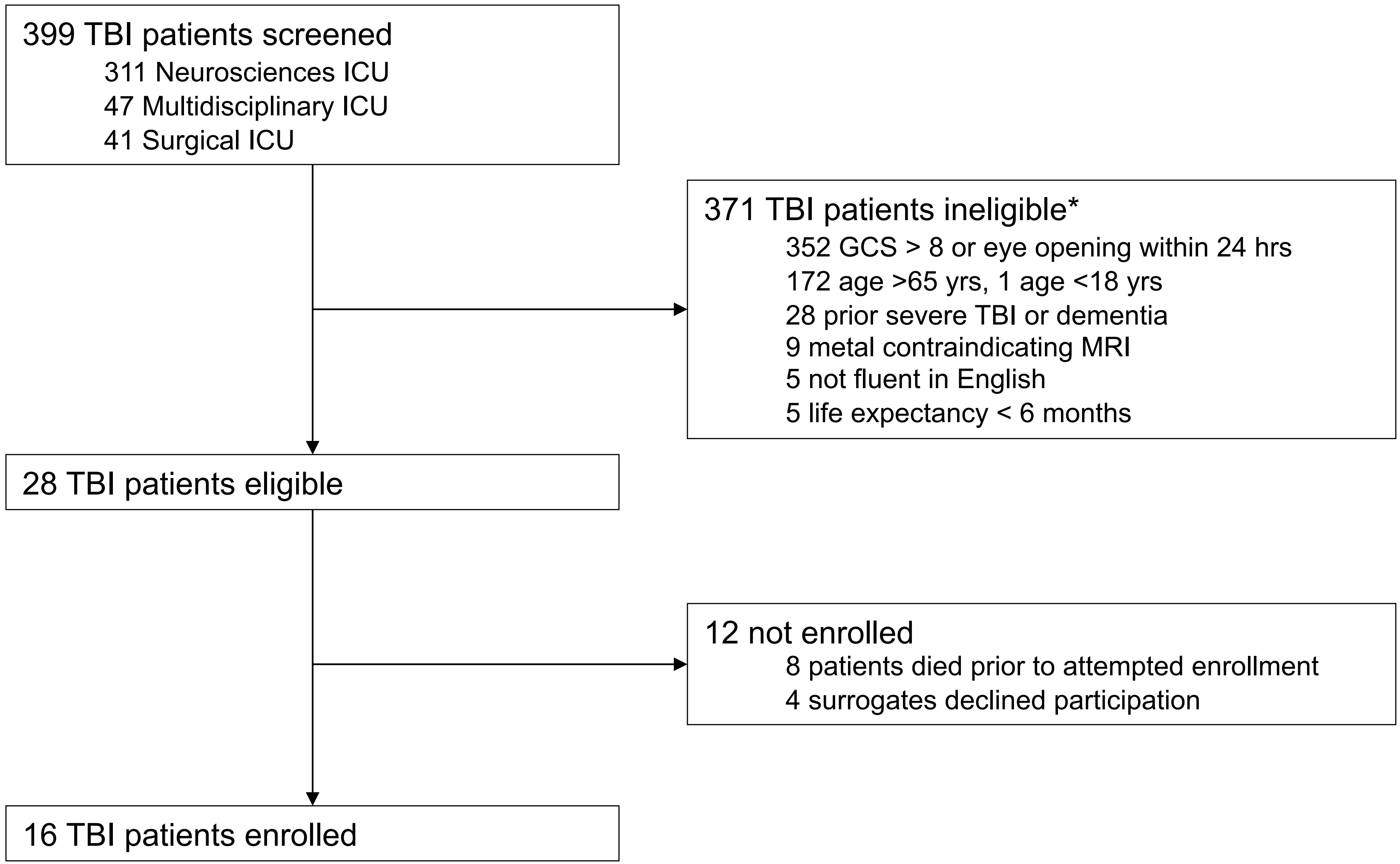
Supplementary Figure 1 Schematic of Language, Music, and Motor Imagery fMRI Paradigms. Each paradigm consisted of two runs of alternating 24-second blocks of stimulus and rest. *For the language paradigm, the second stimulus was backwards (i.e. time-reversed) language.



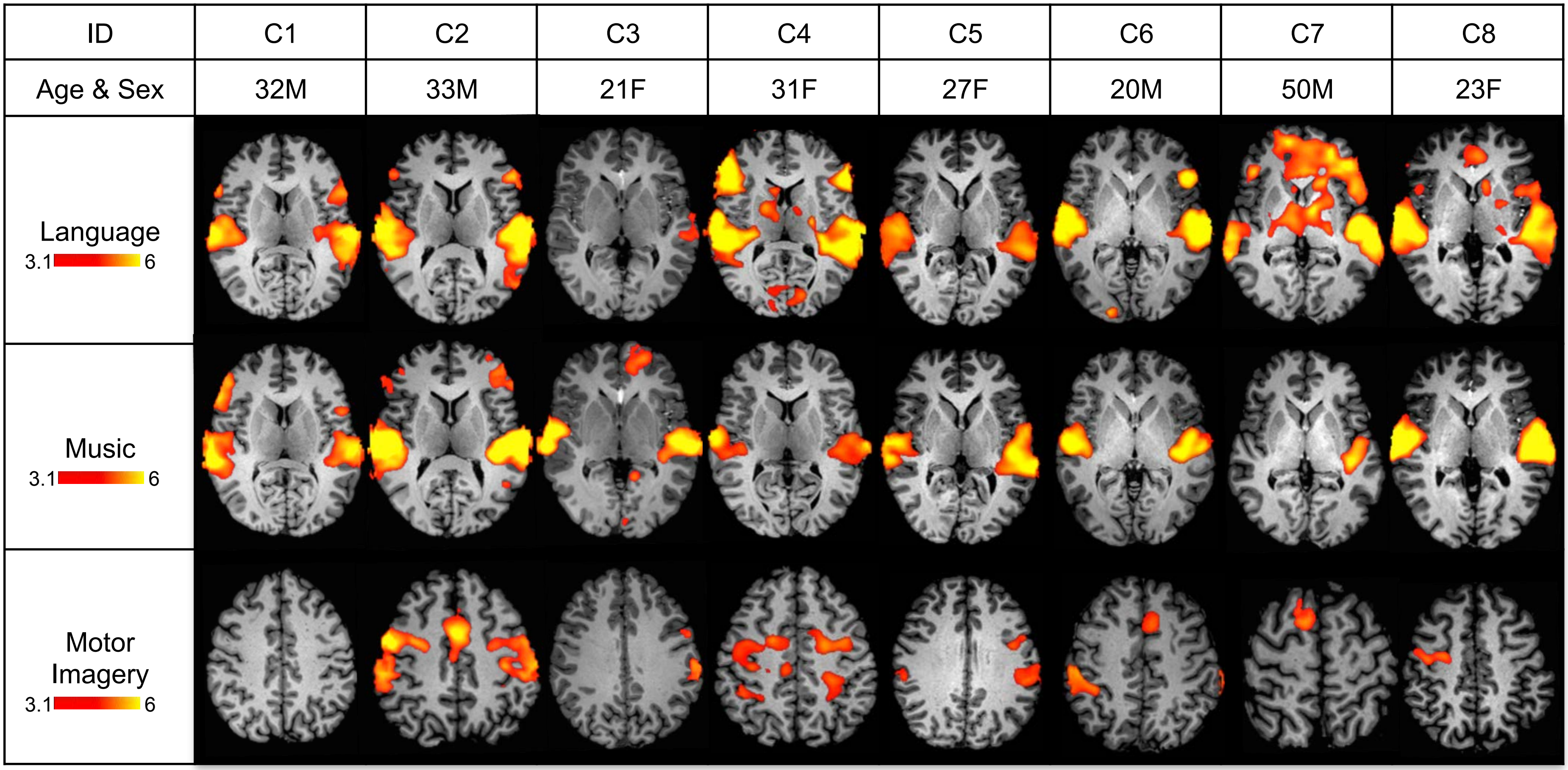
Supplementary Figure 2 Brodmann area 6 region of interest for measuring motor imagery fMRI activation. Superior view of the Brodmann area 6 region of interest (ROI) distributed with the Juelich Histological atlas. This ROI, which was used to measure motor imagery fMRI responses, contains the supplemental motor area (SMA), pre-SMA, and the four components of the bilateral premotor cortices: premotor dorsal rostral (PMDr), premotor dorsal caudal (PMDc), premotor ventral rostral (PMVr), and premotor ventral caudal (PMVc).



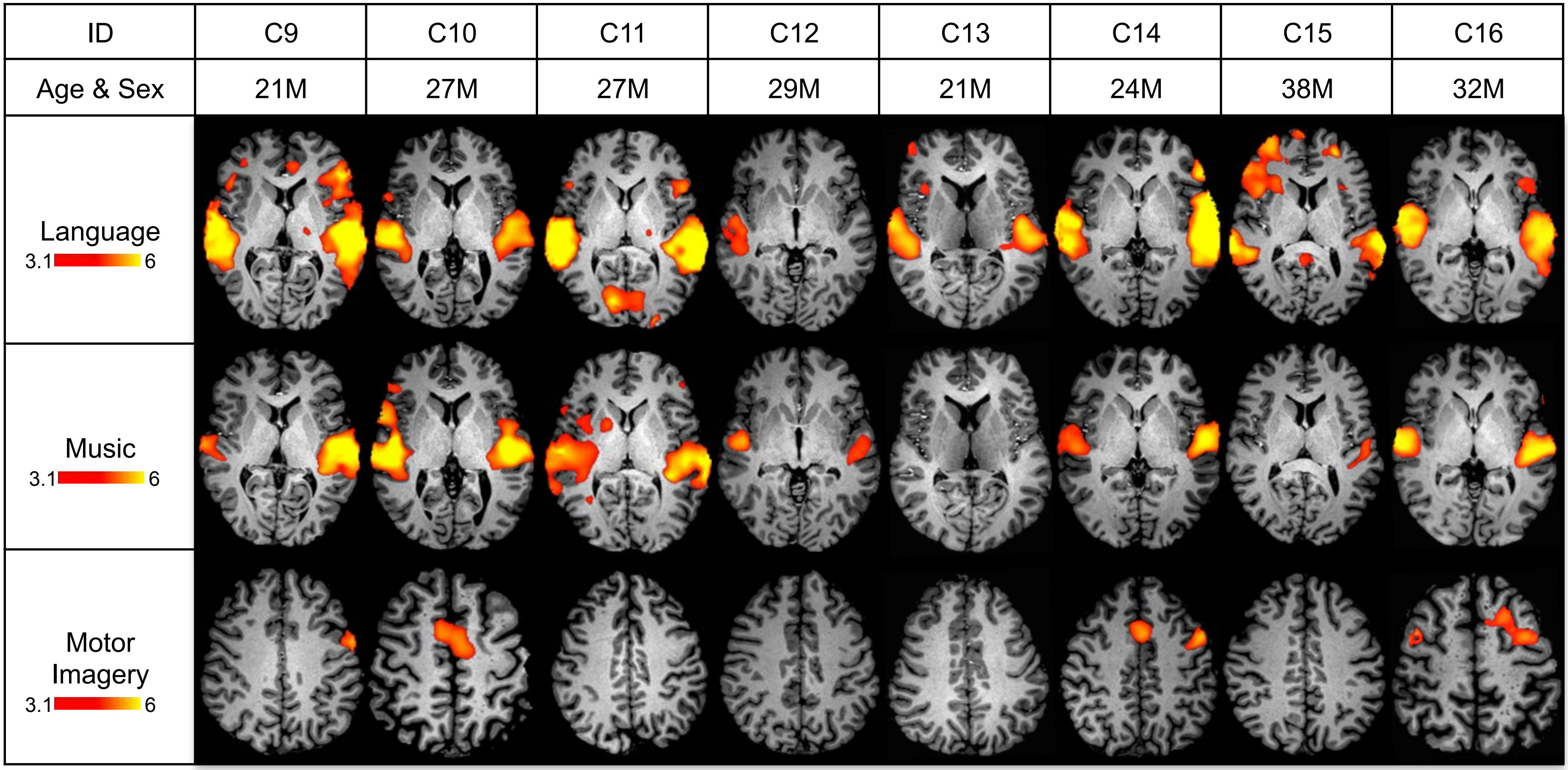
Supplementary Figure 3 Schematic of stimulus-based EEG language, music, and motor imagery paradigms. Each paradigm consisted of 12 alternating 24-second blocks of stimulus and rest.



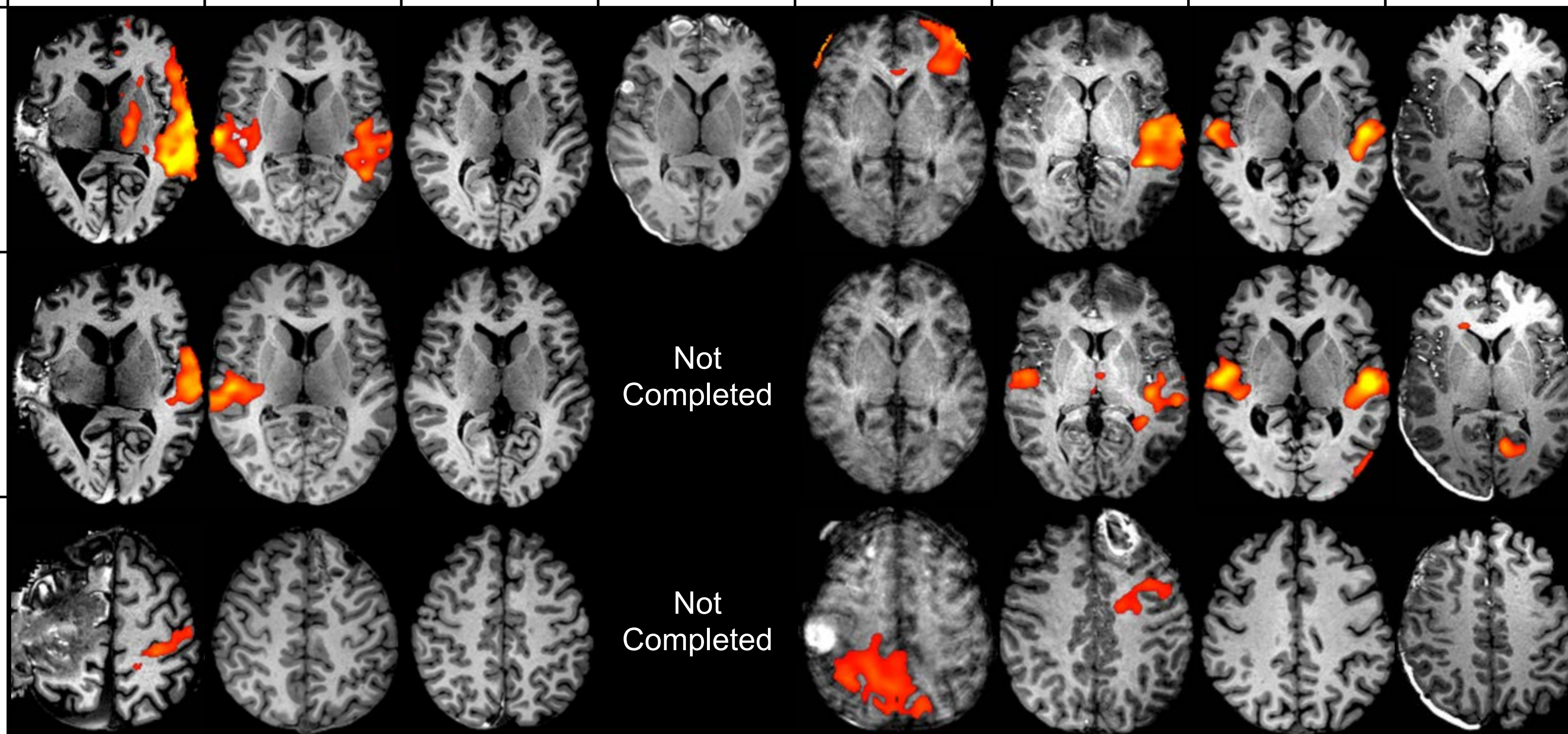
Supplementary Figure 4 Patient screening and enrollment. *Some patients were ineligible for more than one reason. Abbreviations: GCS = Glasgow Coma Scale; ICU = intensive care unit; MRI = magnetic resonance imaging; TBI = traumatic brain injury.



Supplementary Figure 5 Stimulus-based fMRI responses to language, music, and motor imagery in healthy subjects. fMRI data are shown as Z-statistic images to demonstrate stimulus-specific responses. Z-statistic images are thresholded at cluster-corrected Z scores of 3.1 (inset colour bars) and superimposed upon T1-weighted axial images. Abbreviations: F = female; M = male.

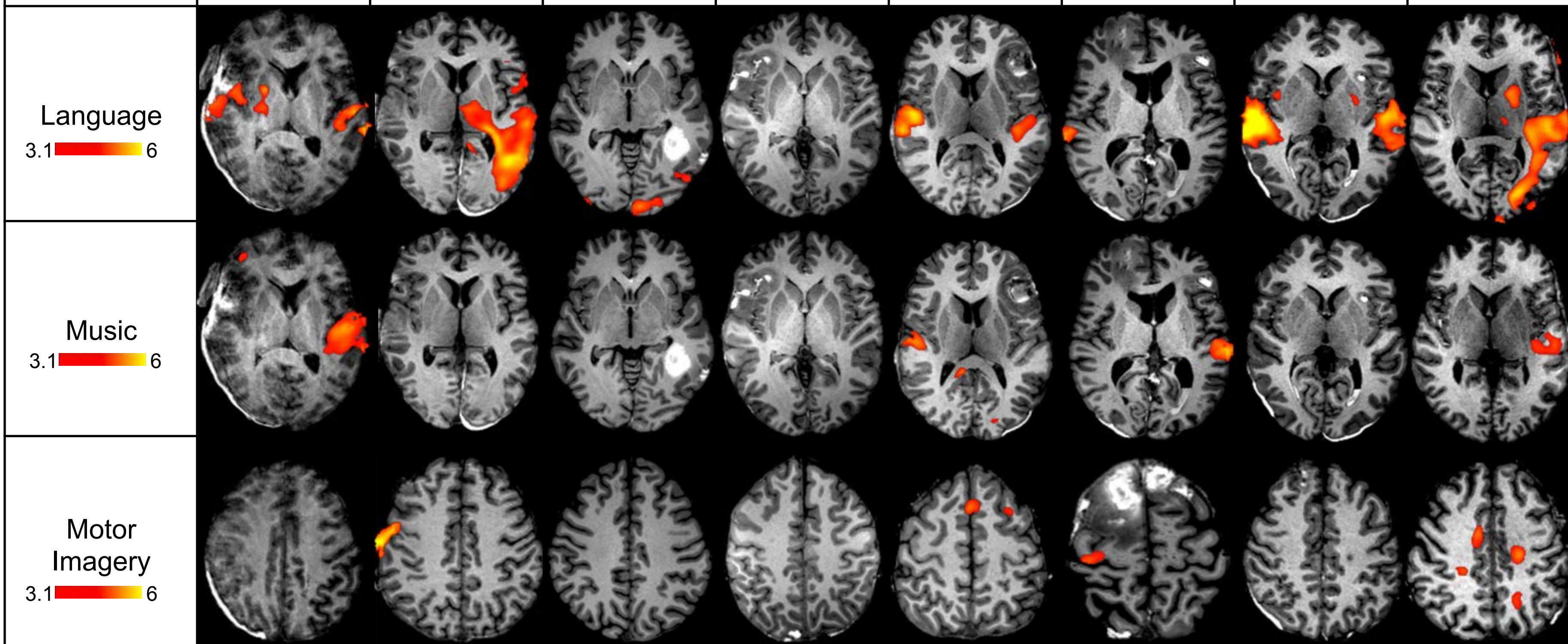


Supplementary Figure 5 (Continued) Stimulus-based fMRI responses to language, music, and motor imagery in healthy subjects. fMRI data are shown as Z-statistic images to demonstrate stimulus-specific responses. Z-statistic images are thresholded at cluster-corrected Z scores of 3.1 (inset colour bars) and superimposed upon T1-weighted axial images. Abbreviations: F = female; M = male.

ID	P1	P2	P3	P4	P5	P6	P7	P8
Age & Sex	27M	21M	19F	19M	34M	28F	45M	33M
iGCS	5T	8	5T	7T	5T	3	5T	5 to 7T
Day of MRI	16	1	3	17	15	7	13	8
LoC at MRI	Oriented	MCS-	Coma	PTCS	VS	VS	MCS+	PTCS
Language 3.1 – 6								
Music 3.1 – 6				Not Completed				
Motor Imagery 3.1 – 6				Not Completed				
GOSE	3	7	7	7	3	3	5	3

Supplementary Figure 6 Stimulus-based fMRI responses to language, music, and motor imagery in patients. fMRI data are shown as Z-statistic images to demonstrate stimulus-specific responses. Z-statistic images are thresholded at cluster-corrected Z scores of 3.1 (inset colour bars) and superimposed upon T1-weighted axial images. The initial Glasgow Coma Scale (iGCS) is defined as the best (i.e. highest) and worst (i.e. lowest) post-resuscitation GCS score prior to ICU admission not confounded by sedation and/or paralytics. Level of Consciousness (LoC) is assessed via behavioural evaluation with the Coma Recovery Scale-Revised (CRS-R) as coma, vegetative state (VS), minimally conscious state without language (MCS-), minimally conscious state with language (MCS+), or post-traumatic confusional state (PTCS). Abbreviations: F = female; GOSE = Glasgow Outcome Scale-Extended; MRI = magnetic resonance imaging; M = male.

ID	P9	P10	P11	P12	P13	P14	P15	P16
Age & Sex	32M	24M	22F	27F	18M	51M	29M	33M
iGCS	5-7T	3-7T	6T	3	3-7	3	4-7	3-4
Day of MRI	11	12	14	8	4	8	7	3
LoC at MRI	MCS+	MCS-	PTCS	Coma	MCS+	VS	MCS-	MCS+



GOSE	4	5	7	1*	7	1*	5	5

Supplementary Figure 6 (Continued) Stimulus-based fMRI responses to language, music, and motor imagery in patients. fMRI data are shown as Z-statistic images to demonstrate stimulus-specific responses. Z-statistic images are thresholded at cluster-corrected Z scores of 3.1 (inset colour bars) and superimposed upon T1-weighted axial images. The initial Glasgow Coma Scale (iGCS) is defined as the best (i.e. highest) and worst (i.e. lowest) post-resuscitation GCS score prior to ICU admission not confounded by sedation and/or paralytics. Level of Consciousness (LoC) is assessed via behavioural evaluation with the Coma Recovery Scale-Revised (CRS-R) as coma, vegetative state (VS), minimally conscious state without language (MCS-), minimally conscious state with language (MCS+), or post-traumatic confusional state (PTCS). * Patient died due to withholding of life-sustaining treatment. Abbreviations: F = female; GOSE = Glasgow Outcome Scale-Extended; MRI = magnetic resonance imaging; M = male; N/A = not applicable.