Supplementary Online Content


**Appendix.** Narrative Clinical Histories and Univariate Analysis Method

**eReferences**

This supplementary material has been provided by the authors to give readers additional information about their work.
Supplementary Materials

Narrative Clinical Histories

Subject 1. The brain-injured subject is a 24-year-old woman who suffered an idiopathic basilar artery occlusion producing multi-focal brain lesions 29 months prior to the fMRI evaluations reported here. The only known risk factor for stroke or other thromboembolic disorders was the use of oral contraceptives. There was no significant past medical history, history of developmental disorders or history of substance abuse. She was completing her senior year of college at the time of onset and had complained of a headache several hours before being found unconscious in her room. Evaluations first at a local hospital and after transfer to an academic medical center diagnosed with basilar artery occlusion. Intraarterial thrombolytic treatment produced reperfusion of the vertebrobasilar circulation but there were residual ischemic lesions in the posterior circulation. She was transferred to a rehabilitation hospital 3 weeks after onset and described as unconscious, with no eye opening. Within the next month she demonstrated partial left eye opening, and within 3 months, partial, bilateral eye opening. At that time moaning vocalizations were noted without facial or limb movements, with the exception of stereotyped, repetitive, left second finger movements. She did not follow commands. At 3 ½ months post-onset she was transferred to a skilled nursing facility. At 4 months post-injury she seemed to demonstrate some context relevant, purposeful behaviors including cry-like vocalizations in response to emotional conversations, humming vocalization in response to singing, and slight movements of her head toward conversations and away from noxious stimulation. A single seizure occurred 7 months after stroke onset.
Elemental neurological examinations have consistently demonstrated: no response to visual threat; bilateral 3rd nerve palsies, more prominent on the right, with nonreactive pupils, right more dilated; bilateral ptosis, with more eye opening on the left; exotropia with almost complete ophthalmoplegia, except for minimal vertical movements on the left. Additional features of the clinical exam include bilateral weak corneal responses, facial diplegia, palatal myoclonus and a strong gag reflex. She has spastic quadriplegia with intermittent extensor posturing.

During her 29 month course post-injury she was treated with amantadine, and/or methylphenidate. Evaluation at nearly 2 years post-onset demonstrated small amplitude vertical left eye movements to command that were shaped into interactive signaling of affirmative responses in a primitive communication system. Responsiveness has been inconsistent and highly dependent on level of alertness.

Primary structural brain injuries included large infarcted territories in the brainstem (ventral pons and tegmental midbrain) and bilateral central thalamic ischemic lesions. Additional areas of ischemic injury included the right calcarine cortex, and bilateral (left greater than right) ischemic injuries to the entorhinal cortices. All vascular lesions were anatomically consistent with loss of arterial blood flow in the basilar artery and bilateral posterior cerebral artery distributions. Structural and metabolic brain imaging revealed
lesions in the anterior intralaminar nuclei (primarily central lateral and paracentral) bilaterally and in the right lateral geniculate nucleus. Resting state fluorodeoxyglucose (FDG) PET data showed notable metabolic downregulation of left hemisphere frontotemporal cortical regions and superior mesial frontal cortices bilaterally consistent with the anticipated projections from the structural lesions in the anterior intralaminar regions.

Quantitative behavioral assessment using the Coma Recovery Scale – Revised (CRS-R) score during the initial evaluation varied during the day. Total CRS-R scores ranged from 9 (auditory 3, visual 3, motor 2, oro/motor 0, communication 0, and arousal 1) on first day of assessment to a maximal measured score of 14 on the following days (auditory 4, visual 5, motor 2, oro/motor 0, communication 1, arousal 2).

Subject 2. A 23-year-old man suffered a severe traumatic brain injury after a motor vehicle accident 23 months prior to this study. The patient was driving his car when he may have skidded on a wet road, running a stop sign and striking a utility pole. He lost consciousness at the scene and was intubated. The initial diagnosis was vegetative state. Four months after injury, the patient showed intermittent interaction with the environment through brief visual fixation and tracking. The patient’s condition continued to improve, and at the time of the study the patient was able to effectively communicate using head movements and a letter board. Using these methods of communication the

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patient had completed an independent evaluation of cognitive function which indicated normal intelligence. The diagnosis at the time of study was locked-in state.

**Subject 3.** A 19-year-old female suffered a severe traumatic brain injury after a motor vehicle accident six months prior to our first study reported here (10 months prior to second study). The patient was riding on the hood of a car when the vehicle made a sudden stop the patient was thrown from the hood of the vehicle suffering head trauma and loss of consciousness. Structural brain imaging revealed multiple minimally displaced fractures and a left middle cranial fossa 2cm epidural hemorrhage. An emergent craniectomy was performed and the hemorrhage was evacuated. Several weeks later, she underwent a right-sided craniectomy for worsening tonsillar and subfalcine herniation. She received a feeding tube and a ventriculo-peritoneal shunt and was later discharged to a neurorehabilitation unit. She returned five months after her injury for a left sided cranioplasty. Following the left cranioplasty the patient showed the first evidence of command following. Our first fMRI assessment occurred just after this time. CRS-R Scores at the time of fMRI assessment revealed a total score of 14 (auditory 4, visual 3, motor 4, oro/motor 0, communication 1, and arousal 2). Three months later, she returned for a right-sided cranioplasty. At the time of our second fMRI evaluation, approximately 10 months after the initial injury, the patient was noted to be intermittently able to communicate and showed improved motor control. CRS-R Scores at the time of fMRI assessment revealed a total score of 19 (auditory 4, visual 4, motor
Subject 4. A 58-year-old woman suffered diffuse strokes and a possible cardiac arrest following knee replacement surgery two years prior to the first studies reported here. Immediately following the surgery, she was observed to be alert and interactive. Forty-five minutes after the surgery, she became suddenly unresponsive, had a generalized seizure, and was intubated. Brain imaging revealed diffuse injury attributed to multiple fat emboli. Patient remained in the intensive care unit for three weeks after which she first opened her eyes and appeared to respond to sound. Later she was transferred to a neuro-rehabilitation unit for 6 weeks, and after about 4-5 months, she began to intermittently exhibit increased awareness and communication.

At the time of the first fMRI studies reported here 20 months after injury the patient’s clinical diagnosis was MCS. She had regained some intermittent speech function. On examination, she was alert and had some occasional simple verbal responses to prompts (i.e. “How are you,” “Fine”), nodded appropriately to yes/no questions, but did not follow commands. She tracked objects and could reach with her right hand. Quantitative behavioral assessment using the Coma Recovery Scale – Revised (CRS-R) score during this evaluation revealed a range of total score of 16-19 (auditory 3, visual [3, 5], motor 5, oro/motor 3, communication [0, 1], arousal 2).
The second clinical evaluation of the patient reported here was performed 32 months after injury. At this time the patient had reestablished consistent interactive verbal communication. She responded accurately to most simple questions and reliably communicated with spoken language. She could clearly recognize family members but remained confused and lacked insight into her deficits. Quantitative behavioral assessment using the Coma Recovery Scale – Revised (CRS-R) score showed a total score of 23 (auditory 4, visual 4, motor 6, oro/motor 3, communication 2, arousal 3). The patient could read and follow written commands but could not write (limited by severe movement impairment and inability to open hand) at this time and could complete assessment using the Mississippi Aphasia Screening Test (MAST) and had total score of 60/100.

**Subject 5.** The patient is a 36-year-old male diagnosed with multiple sclerosis 5 year prior to our evaluation. The patient has had a progressive course of MS with a stepwise decline following the development of a large central pontine area of plaque formation and presentation of acute coma at onset. Over the past four years the patient has remained only intermittently responsive with a diagnosis of minimally conscious state.

Quantitative behavioral assessment using the Coma Recovery Scale – Revised (CRS-R) score at the time of fMRI evaluations demonstrated total CRS-R scores ranging from
10-13 (auditory [0, 2], visual 3, motor [5, 4], oro/motor [0, 1], communication 0, and arousal 2).

**Univariate Analysis Methods**

Univariate analysis was performed for all normal and clinical subjects who performed the command following (CF) and multiple choice (MC) paradigms using the FMRI Expert Analysis Tool (FEAT), v. 5.92, part of the FSL software package (Smith et al., 2004). Statistical parametric maps (SPMs) were computed using FILM in FSL. For the MC condition, the analysis was blinded so that the investigator determining a subject’s answer to the question did not know the correct response. The following preprocessing was applied: motion correction, non-brain removal, spatial smoothing, grand-mean intensity normalization, local autocorrelation correction, and high-pass temporal filtering (32 s cut off). Z-statistic images were thresholded using clusters determined by Z > 2.3 and a multiple-test-corrected cluster-significance threshold of p = 0.05 (Worsley et al., 1996). These statistical parametric maps were computed for each potential response, which were each then interrogated in a hierarchical fashion. First, the statistical maps were analyzed for significant activation in the brain area recorded during the CF or, in the cases where CF was omitted, the BC task. For all normal subjects, this area was congruent with the supplementary motor area (SMA) as defined by the Harvard-Oxford Cortical Atlas. If no area or multiple areas appeared significantly active in SMA, the time course and overall brain activity were analyzed for evidence of event-related activity within a larger region of interest.
Analysis was also performed using BrainVoyagerQX version 1.10.4.1250 (Goebel et al., 2006). This analysis was used for the production of figures for the manuscript; however, all results match those of the FSL analysis above. For all tasks, volumes of time series were corrected for slice scan-time differences and for motion, spatially filtered with a 3D Gaussian smoothing kernel, corrected for linear trends, and high-pass filtered with a cutoff of 3 cycles over the whole time span. The time-series volumes were then co-registered to the 3D anatomical volumes, and these were normalized to Talairach coordinates. For the computation of statistical parametric maps, general linear models were used (Friston et al., 1995), taking the six motion-correction-parameter time-series into account as nuisance variables, which were regressed out. The hemodynamic response function was modeled as a gamma function (we found that for the shorter block lengths of 16 s as described above the data was better modeled than with using the difference of two gamma functions). The results of all single-subject and group analyses were thresholded using the false-discovery rate (FDR), which was calculated for \( p < 0.05 \) (Benjamini and Hochberg, 1995; Genovese et al., 2002). The group analyses of the CF and BC tasks were fixed-effects analyses.
References


