SUPPLEMENTARY MATERIALS

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Supplementary data

1. Clinical history

Subject 1: The subject was a typically developing 9-year-old when he experienced a traumatic brain injury as a restrained passenger in a motor vehicle collision. He was intubated at the scene with a Glasgow Coma Scale (GCS) of 4. The initial studies demonstrated a severe traumatic brain injury with diffuse axonal injury (DAI). The patient was taken to surgery for stabilization of bone fractures (unspecified in available record). Following the surgery and while being transported from the operating room back to the recovery room, the patient "became increasingly more difficult to ventilate with a bag". Heart rate decreased to as low as 50, at which time cardiopulmonary resuscitation (CPR) was initiated. He was provided approximately 7 to 10 minutes of chest compressions and received 2 doses of epinephrine. He was reintubated, at which time a clot was found in the old endotracheal tube and thought to be the likely cause of his acute onset of hypoxemia. The patient was then returned to the pediatric intensive care unit. He was thought to have suffered an hypoxic ischemic event with ensuing encephalopathy. The next day, an external ventricular drain was placed to monitor intracranial pressures. No further comments on level of consciousness exist in the record.

Upon discharge, 8 months after initial admission, the patient was given a principal diagnosis of anoxic brain injury and the following secondary diagnoses: 1. Traumatic brain injury. 2. Paroxysmal autonomic instability and dystonia (PAID) syndrome.3. Feeding difficulties.4. Central sleep apnea.5. Osteopenia.6. Hypercalcemia. 7. Cardiopulmonary arrest.8. Respiratory failure.9. Fractures (right humerus; right distal femur; left femoral neck; compression fractures of T5,T6, T7, T8, and T9; metacarpals 3, 4, and 5 of the right hand; metatarsals of the right

foot;multiple pelvic fractures).10. Deep venous thrombosis of the right axillary, subclavian, and brachial veins.11. Grade IV spleen laceration.12. Contusion, left adrenal gland.

CT scan of the head w/o contrast (day of injury):

1. Small areas of supratentorial parenchymal hemorrhages as detailed. Left frontal region has a contusion.
2. Right inferior frontal findings may represent contusion and/or subtle/subarachnoid blood
3. products with adjacent edema.
4. No calvarial or cervical spine fracture identified on the 3D reconstructions. There is
5. straightening of the normal cervical lordosis.
6. Findings suspicious for a radiopaque foreign body within the medial/anterior aspect of the right orbit, along the anterior margin of the right lobe. A calcific density anterior to the left ear is favored, chronic in nature given its configuration.

MRI of the brain (one day after injury)

1. Mild-to-moderate burden of scattered hemorrhage and ischemic white matter shear injury.
2. Bilateral basal ganglia and right posterior cingulate gyrus late acute mild ischemia.
3. MRI of the brain w and w/o contrast (3.5 months after injury):
4. Multi focal findings compatible with the sequelae of prior traumatic brain injury including encephalomalacia, global volume loss, probable laminar necrosis, DAI, and ischemic injury.

5. Periventricular signal changes, possibly related to prior increased intraventricular pressure.
6. Small, nonspecific, scattered foci of T2 hyperintensity likely also related to prior trauma.
7. Ventriculomegaly related to global volume loss, similar in appearance to prior examination

EEG (one day after injury)

1. Findings are abnormal due to synchronous and asynchronous episodes of voltage attenuation, which resolved by the end of recording, indicating severe disturbances of function involving both hemispheres.
2. During brief periods of wakefulness, there is intermittent slow (delta) activity over the left
3. temporal region, indicating focal disturbance of function involving this region.
4. Absence of spindles, indicating disturbance of thalamocortical pathways.
5. Multifocal epileptiform discharges, most prominently over the left central parietal region.
6. No electrographic seizures were recorded. Overall, these findings are consistent with an underlying encephalopathy, which improved over the course of recording. They also indicate a propensity for partial onset seizures.

EEG (3 months post injury):

1. An abnormal EEG recording, obtained in a clinically lethargic state. Background activity shows low amplitude slow features, with potentially a greater degree of background suppression over the left side of the head in comparison to the right.

2. Evidence of reactivity to external stimuli is observed. There are no epileptiform abnormalities, and no evidence of seizure activity. These EEG features are consistent with an encephalopathic state of either diffuse or multifocal origin, potentially affecting the left side to a greater degree.

EEG (7 months post injury):

1. Abnormal EEG recorded in the awake state alone. This recording has a poorly organized and poorly sustained EEG background composed entirely of low amplitude, high frequency beta with poor signal to noise quality. This is best seen over the right posterior quadrant and less well-developed over the left posterior quadrant.
2. Elsewhere, the background is largely obscured by muscle artifact.

Subject 2: Parents reported that the patient, a typically developing 13 year old, had experienced palpitations with exertion over several months and had been evaluated by a cardiologist a few weeks prior to the incident. A normal echocardiography and electrocardiogram were noted. On the day of the event, the subject reported feeling unwell during gym class and left to use the bathroom. When he did not return, staff found him 5 minutes later unresponsive. CPR was initiated with medics arriving after 3 minutes. Automated external defibrillator was placed and four separable shockable rhythms were noted during shock administration with intermittent episodes of CPR. IV epinephrine was administered during the approximately 30 minutes prior to emergency room (ER) arrival. Medics reported that the patient had returned to spontaneous circulation 2 minutes prior to ER arrival. The patient was intubated and IV access was obtained in the field during pediatric advanced life support. CPR is reported to have lasted 30 minutes.

Lack of pupillary reactivity noted at ER. Patient was resuscitated in the ER and transferred to the pediatric intensive care unit where he continued to require further resuscitation. Due to worsening respiratory status, despite being on maximal ventilator support, he was transferred to a University Hospital for cardiac ablation and implantation of a loop recorder. He was intubated for 3 weeks and spent several months a University Hospital. By family report, patient received hypothermia but no existing records confirm duration.

Upon discharge, the primary diagnosis was anoxic brain injury following cardiac arrest resulting in global developmental delay and spasticity. No comment on level of consciousness.

CT without contrast (Day of injury):

1. No significant atrophy.
2. No focal intracranial lesion.
3. No mass, hemorrhage, or hydrocephalus.

MRI without contrast (7 months post injury)

1. Loss of brain parenchymal volume since prior CT scan. There is no prominence of the sulci and ventricular system.
2. No acute ischemic injury is identified on diffusion imaging.
3. Increased T2 signal is noted in the pons and cerebral peduncles and vague increased T2 signal in the cerebral white matter.

4. Although the atrophy is not rather diffuse it appears to be somewhat more prominently involves the pre and postcentral gyri. Some cortical high signal is also noted along the postcentral gyri, left greater than right.

EEG (7 months post injury)

1. The background is diffusely attenuated and undifferentiated. The background consists primarily of low amplitude alpha, theta, and beta frequencies, which are not well organized.
2. No clear posterior dominant rhythm noted.
3. No epileptiform activity was noted.

2. Methods

a. EEG data preprocessing and event related potential (ERP) analyses

EEG data were analyzed using EEGLAB[1] in MATLAB (The MathWorks, Inc). EEG signals were high-pass-filtered at 1Hz before 60Hz line noise removal, and downsampling to 200 Hz. The artifact subspace reconstruction method [2] was further applied to remove transient high-amplitude artifacts from the continuous EEG data. Eye blinks and muscle contraction artifacts

were removed by rejecting the corresponding sources from an independent component analysis (ICA) decomposition using the Infomax algorithm[3] and projecting back into the sensor space.

We derive two event-related potential (ERP) measures at the vertex electrode: auditory evoked potential. After preprocessing, trials were segmented into 1100ms epochs time-locked to the onset of the auditory stimulus (100ms pre-stimulus and 1000ms post-stimulus). Analysis then followed standard procedures[4]: each trial was baseline-corrected (by subtracting the mean voltage over the 100ms pre-stimulus interval), low-pass filtered at 20 Hz, and averaged time-locked to the stimulus onset. For the analysis of auditory evoked potentials (AEPs) this used standard tones only; for the oddball response analysis, a difference wave was computed (average response to deviant stimuli of either kind, minus average response to standard tones). We corrected this for the effect of noise by computing a signal-to-noise ratio (SNR), i.e. by dividing the mean voltage signal by its own trial-to-trial standard deviation at each time sample. For the difference waves, this standard deviation was computed as the square root of the sum of the trial-to-trial variances of the deviant-class amplitudes and standard-class amplitudes.[5] To obtain a single number reflecting each participant's AEP, whose latency varied according to age,[6] we computed the largest negative value of the SNR at electrode location Cz in the interval 60–260 ms following stimulus onset. In the oddball difference waveforms, we computed the largest positive SNR at Cz in the interval 200–450 ms to capture the P3 component.[7]

b. Spectral analysis of motor-command-following EEG

After preprocessing, the EEG data were divided into 9-second trials, each trial starting 3s after the end of the auditory cue. The trials were separated and grouped by condition (move left: n=8; move right: n=8; rest: n=16); Signals were spatially filtered using a a Hjorth Laplacian montage,[8]

and power spectral density estimates were obtained using a multi-taper method with 5 tapers, resulting in a frequency resolution of 2 Hz. As in previous work,[8] we used the two-group test[9] to determine statistical significance of power spectral differences at each electrode (Chronux toolbox for Matlab, www.chronux.org) employing a jackknife method with a cutoff of $p \leq 0.05$ before false-discovery-rate correction. Significant separations between hand movement and rest conditions in the alpha or theta frequency band) power were taken as evidence of a response. Furthermore, we boiled down the question of a positive or negative outcome to two statistical tests per participant—one for attempted left-hand movement and one for right. For each, we computed the signed coefficient of determination between the bandpower values at each electrode, and a binary variable indicating the instruction (rest or movement), and then subtracted the sum of the values in contralateral electrodes from the sum of the values in ipsilateral electrodes. A positive net value would be expected in successful command following, as it indicates consistent contralateral event-related desynchronization and/or ipsilateral event-related synchronization;[10] a negative value would indicate the reverse. The significance of the statistic was assessed using a one-sided permutation test in which the labels (rest and movement) were randomly reassigned to the trials in each of 1000 repetitions. We considered a participant to have a positive response if either the left-hand or right-hand p-value was equal to or less than 0.05.

Supplementary table 1

	Subject 1	Subject 2	Interpretation of positive findings
Behavioral assessment (CRS-R Total)	10 (initial visit), 7 (one year follow-up)	6	N/A
PET, metabolism	Disproportionately decreased FDG avidity in the bilateral frontal	Disproportionately decreased FDG avidity in the bilateral parietal	N/A

	cortex.	precuneus, left greater than right lateral parietal convexity, and bilateral calcarine cortex.	
MRI, structure	Moderate frontal predominant parenchymal volume loss.	Generalized parenchymal volume loss with superimposed disproportionate volume loss in the left frontal, temporal and parietal operculum.	N/A
fMRI, command-following	Differential response (hand movement attempts vs. rest) in bilateral premotor cortex ($p < 0.005$, uncorrected); Differential response (imagined spatial navigation vs rest) at the occipito-parietal junction ($p < 0.001$, uncorrected).	No significant responses were observed to either command type.	Demonstrates ability to distinguish one verbal command from another and consistently associate commands with the correct respective responses; ability to sustain attention and focus on voluntary actions over several minutes.
EEG, command-following	Event-related desynchronization of contralateral sensorimotor rhythm during attempted movement of the left hand ($p = 0.04$) but not right ($p = 0.67$).	Event-related desynchronization of contralateral sensorimotor rhythm during attempted movement of either hand (left: $p = 0.01$; right: $p = 0.03$).	
EEG, response to tones	Larger auditory evoked potentials (AEP) than in DoC subjects without positive command-following.	Larger auditory evoked potentials (AEP) than in DoC subjects without positive command-following.	In combination with P300, may be a biomarker of cognitive state.
EEG, contrast frequent standard tones with rarer "oddball" sounds	Larger P300 responses in the oddball difference wave, than in DoC subjects without positive command- following.	Larger P300 responses in the oddball difference wave, than in DoC subjects without positive command- following.	Correlates with mechanisms of orienting attention. In combination with AEP, may be a biomarker of cognitive state.
EEG, contrast congruent vs. incongruent	Significant N400 event-related potential ($p < 0.05$) with latency and morphology typical	Significant N400 event-related potential ($p < 0.05$) with latency and morphology typical	Reflects processing of the meaning of spoken words in context.

endings in spoken sentences	of uninjured subjects.	of uninjured subjects.	

Supplementary references

1. Delorme A, Mullen T, Kothe C, et al. EEGLAB, SIFT, NFT, BCILAB, and ERICA: New Tools for Advanced EEG Processing. Computational Intelligence and Neuroscience. doi:https://doi.org/10.1155/2011/130714
2. Mullen TR, Kothe CAE, Chi YM, et al. Real-time neuroimaging and cognitive monitoring using wearable dry EEG. *IEEE Trans Biomed Eng*. 2015;62(11):2553-2567. doi:10.1109/TBME.2015.2481482
3. Bell AJ, Sejnowski TJ. An information-maximisation approach to blind separation and blind deconvolution. *Technology*. 1995;1159:1129-1159. doi:10.1162/neco.1995.7.6.1129
4. Luck SJ. *An Introduction to the Event-Related Potential Technique*. MIT press.; 2014.
5. Heinrich SP, Bach M. Signal and noise in P300 recordings to visual stimuli. *Doc Ophthalmol*. 2008;117(1):73-83. doi:10.1007/s10633-007-9107-4
6. Ponton CW, Eggermont JJ, Kwong B, Don M. Maturation of human central auditory system activity: evidence from multi-channel evoked potentials. *Clin Neurophysiol*. 2000;111(2):220-236. doi:10.1016/S1388-2457(99)00236-9
7. Polich J. Updating P300: An Integrative Theory of P3a and P3b. *Clin Neurophysiol Off J Int*

Fed Clin Neurophysiol. 2007;118(10):2128-2148. doi:10.1016/j.clinph.2007.04.019
8. 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 Off J Int Fed Clin Neurophysiol*. 2011;122(11):2157-2168. doi:10.1016/j.clinph.2011.03.022
9. Bokil H, Purpura K, Schoffelen JM, Thomson D, Mitra P. Comparing spectra and coherences for groups of unequal size. *J Neurosci Methods*. 2007;159(2):337-345. doi:S0165-0270(06)00328-1 [pii] 10.1016/j.jneumeth.2006.07.011
10. Pfurtscheller G, Lopes da Silva FH. Event-related EEG/MEG synchronization and desynchronization: basic principles. *Clin Neurophysiol*. 1999;110(11):1842-1857. doi:10.1016/S1388-2457(99)00141-8