Supporting Online Material for

Detecting Awareness in the Vegetative State

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This PDF file includes:

Supporting online material.

Materials and Methods

Patient

To examine neural responses to aurally-presented sentences, a sparse imaging technique was used to minimize interference from scanner noise. The patient was played a single sentence (or noise-equivalent) in the 7.4s silent period before a single 1.6s scan with stimulus timing jittered relative to scan onset. There were 118 spoken sentences trials, 59 signal correlated noise trials, and an additional 60 silent trials for the purpose of monitoring data quality. The signal correlated noise stimuli had the same duration, spectral profile and amplitude envelope as the original speech, but were entirely unintelligible (S1). The experiment was divided into three sessions of 79 trials with events pseudo-randomly ordered within each scanning session. The sentences were presented using a MRI compatible auditory stimulus-delivery system (Resonance Technology, Northridge, CA), with insert earplugs to further attenuate scanner noise. DMDX software running on a Windows XP PC was used to present the stimulus items.

The mental imagery task was administered as a block design with alternating 30-second periods of task and rest. Pre-recorded spoken instructions ("imagine playing tennis", "imagine visiting the rooms in your home", "now just relax") were given at the start of each block. Each imagery task and the rest condition were repeated 10 times.

For both fMRI studies, statistical parametric mapping software (SPM2) was used to identify task-specific activation at the single subject level. Data were first manually reoriented. The mean image of the realigned scans were computed and used as source image for spatial normalisation of the data. Data were then smoothed using a 12 mm FWHM Gaussian isotropic kernel. All results were thresholded at FDR corrected $p<.05$.

Signed assent from the patient's next of kin was acquired prior to investigation. This study was approved by the Cambridge Local Research Ethics Committee.

Healthy Volunteers

To assess replicability and specificity at the single-subject level, the two mental imagery tasks were also administered to 34 healthy volunteers at three separate imaging centres (Wolfson Brain Imaging Centre, Cambridge, UK, Bruker 3T MR, n =12, see Fig S2; Cyclotron Research Centre, Liège, Belgium, Siemens Allegra 3T MR, n=12, MRC Cognition and Brain Sciences Unit, Cambridge, UK, Siemens TIM Trio 3T MR, n=10) using a formally identical procedure to that used to assess the patient. For each study, statistical parametric mapping software (SPM2) was used to identify task-specific activation at the single subject level. Preprocessing was identical to that described above for the patient. Random effects analyses identified areas active for each mental imagery task relative to rest. All results were thresholded

at FDR corrected $p<.05$. Imagining playing tennis elicited activity in the supplementary motor area in all 34 volunteers scanned and imagining moving from room to room in the house activated the parahippocampal cortices, the parietal and lateral premotor cortices, bilaterally.

For comparative purposes, signal intensity changes for the patient in these four regions were plotted against those of 12 healthy volunteers scanned at the Wolfson Brain Imaging Centre, Cambridge, while performing exactly the same two tasks (Fig. S2). In all four regions, the activity observed in the patient was within the normal range of responses. Although it is theoretically possible that the mere instruction to imagine such actions triggers specific and automatic changes in brain activity, the complexity of the commands used here and the richness of the imagery that is likely to be required to produce a response that is indistinguishable from that of healthy individuals (Fig. S2), make this possibility extremely unlikely. Signed consent from all volunteers was acquired prior to investigation.

Clinical history and additional investigations

In July 2005, the patient was involved in a road traffic accident. On admission to hospital she had a Glasgow Coma Scale score of 4 (S2). A computed tomography (CT) scan revealed diffuse brain swelling, intraventricular blood in the left lateral ventricle, low attenuation in the left frontal lobe close to the corpus callosum and attenuation change in the right frontal and left posterior temporal regions. The following day she underwent a bifrontal decompressive craniectomy and a month later a ventriculoperitoneal shunt was inserted into the right lateral ventricle. Between the time of the accident and the fMRI scan in January 2006, the patient was assessed by a multidisciplinary team employing repeated standardized assessments consistent with the procedure described by Bates, 2005 (S3). Throughout this period the patient's behaviour was consistent with accepted guidelines defining the vegetative state (S4). She would open her eyes spontaneously, exhibited sleep/wake cycles and had preserved, but inconsistent, reflexive behaviour (startle, noxious, threat, tactile, olfactory). Her response to noxious stimuli was hard to elicit and her olfactory response was inconsistent. No elaborated motor behaviours, which are regarded as 'voluntary' or 'willed' responses (S5), were observed from the upper or lower limbs. There was no evidence of orientation, fixation greater than 5 seconds or tracking to visual or auditory stimuli. No overt motor responses to command were observed. During this period of assessment, the Wessex Head Injury Matrix (S6) was administered repeatedly at different times of the day and in different postural positions. The highest ranked behaviour over a 5-day assessment period, including the day of the fMRI scan, was 13 (of 62) and the total number of behaviours observed was 7 (of 62). A structural MRI (axial T2 and proton density) was performed at the time of the fMRI scan. The brainstem appeared to be intact. The frontal part of the brain was partially sunken in as the result of the bifrontal decompression. Both lateral ventricles were well drained with the right being slightly larger than the left corresponding to the sulcal atrophy in the right lateral frontal and right anterior temporal regions and the deep white matter hyper-intensity in the right frontal lobe. The susceptibility artefact created by the large bifrontal craniotomy precluded detailed assessment of the inferomedial frontal regions. Electroencephalography showed a symmetrical mixed background record consisting of predominately theta (4-7Hz up to 80μV) and some slow alpha frequencies (8-10Hz up to 35μV). Sparse beta frequencies were superimposed. Underlying delta frequencies (1-3Hz up to 120μV) were also detected. A breach rhythm was detected over the craniectomy wound. The EEG showed a reflexive response to a loud auditory stimulus only. No sustained period of eye opening was observed during the assessment period

and so it was unclear whether alpha frequencies were attenuated by eye opening. The brainstem auditory evoked potential showed a preserved response from the eighth cranial nerve, pons and midbrain bilaterally. Onset latencies were within normal limits. The somatosensory evoked potential showed a preserved primary (N20), secondary (P27) and association cortex (N30) response (right hemisphere) following left median nerve stimulation only. Onset latencies were within normal limits. It was not possible to obtain a response from the left hemisphere due to technical difficulties. A flash evoked visual potential using LED-goggles produced a distinguishable N1-P1 and N2-P2 response bilaterally. Onset latencies were delayed bilaterally. Transcranial magnetic stimulation (TMS) suggested that the motor pathways in the upper limbs were preserved bilaterally, although conduction delays were observed to right abductor policis brevis. Throughout the assessment and the imaging procedures described below there were no discernible changes in behaviour to indicate discomfort or pain.

Outcome and diagnostic distinction

International guidelines, including those of the Royal College of Physicians (S4) in the U.K. and the Multi-Society Task Force (S5), representing five major medical societies in the U.S. suggest that a diagnosis of permanent vegetative state should not be made in cases of traumatic brain injury until 12 months post injury. It is important to note that recovery from post-traumatic vegetative state at six months remains nearly 20% for recovery of consciousness with a quarter of those recovering, moving on to an independent level of function. In contrast to the patient described here, a vegetative state originating from an anoxic brain injury may be considered permanent at 6 months post injury. Non-traumatic injuries are considered to have a poorer prognosis. At the time of writing (11.5 months post injury), the patient described here was clinically re-examined. In response to a mirror held in front of her, which was then slowly moved to 45 degrees on either side, she turned her eyes very slowly to the right, but not the left, on two trials and fixated for more than five seconds. Thereafter, there was no response to the mirror and no response to any other object, either before or after the trials with the mirror. There was no response to a noxious stimulus except for a transient small dilatation of the left pupil and no response to command.

Supporting figures

Figure S1

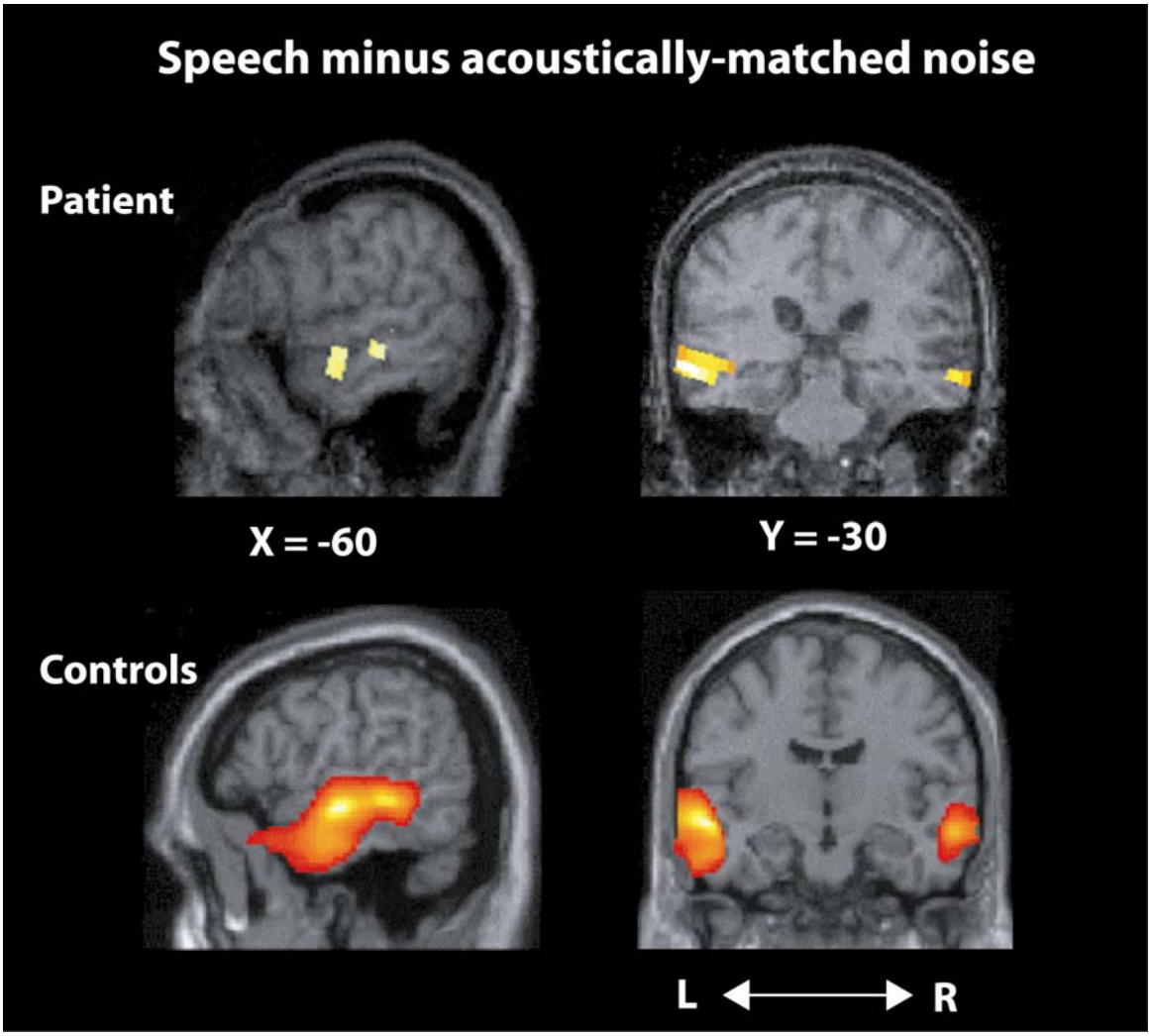

Figure S1 Caption
Superior and middle temporal gyri response to hearing sentences versus signal correlated noise in the patient and in a group of healthy volunteers in sagittal (left) and coronal (right) planes. All results are thresholded at FDR $p<.05$, corrected for multiple comparisons. In this figure and in Fig. 1 (see main text) control group activity is superimposed on the standard canonical T1-weighted structural MRI and the patient activity is superimposed on her own normalized T1-weighted structural MRI on which damage to the frontal cortex can clearly be seen. Healthy volunteer data reported previously (S1).

Figure S2

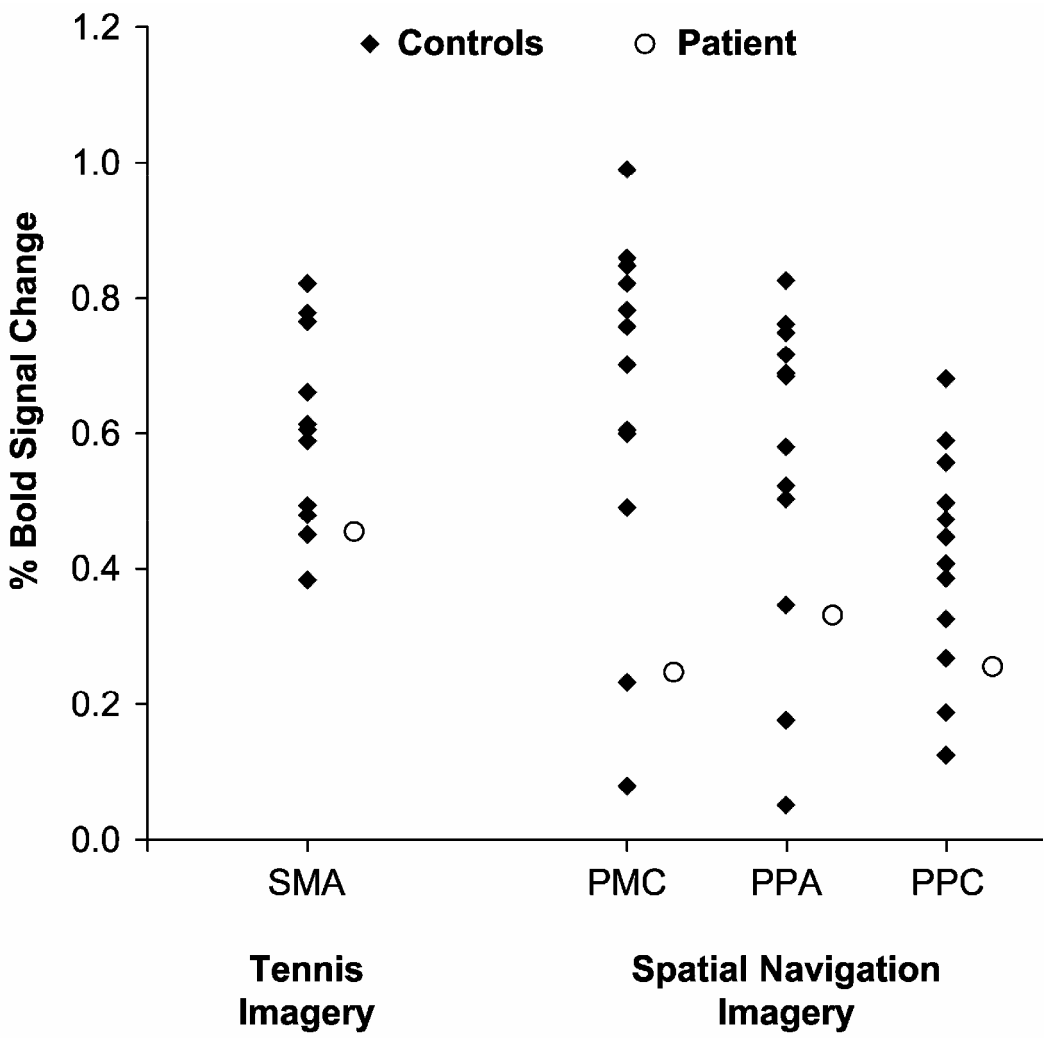

Figure S2 Caption
Signal intensity changes in the patient plotted against those of 12 healthy volunteers performing the same two tasks. In all four predicted regions (supplementary motor area; SMA, parahippocampal gyrus; PPA, posterior parietal-lobe; PPC, lateral premotor cortex; PMC) signal intensity changes in the patient are within the normal range.

Supporting references

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S6. A. Shiel *et al*., *Clinical Rehabilitation* **14**, 408 (2000).