Supplementary Online Content


eMaterial

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This supplementary material has been provided by the authors to give readers additional information about their work.
Narrative Clinical Histories

Patient 1. The patient suffered a cardiac arrest from being kicked in the chest, in August 1997. The hypoxic event led to secondary hypoxic ischemic encephalography with multiple neurological deficits. After a three-week coma, the patient received a diagnosis of VS and was discharged to a long-term care facility. In six behavioral assessments conducted by the research team, in the three months prior to the fMRI testing, the patient scored 6-10 (out of a maximum of 23) in the JFK Coma Recovery Scale (CRS-R). In 4/6 of these assessments he displayed evidence of visual pursuit, consistent with a diagnosis of MCS. Similarly, when admitted for fMRI testing, 184 months post injury, the patient scored 9/23, and received a diagnosis of MCS. (CRS-R subscores: auditory: 1 – startle; visual: 3 – visual pursuit; motor: 2 – flexion withdrawal; oromotor/verbal: 1 – oral reflexive movement; communication: none; arousal: 2 – eye opening w/o stimulation.)

Patient 2. The patient sustained a traumatic brain injury as a result of a motor vehicle accident, in March 2007. He suffered prolonged loss of consciousness, attributable to bilateral intracerebral haemorrhage (with ventricular extension), as well as poly trauma. Following the injury, he required intensive care, including external ventricular drain placement, by neurosurgery. A few months after the injury, he was discharged to a rehabilitation program with a diagnosis of MCS. Subsequently, in the three years prior to scanning, the patient was cared for at home. In seven behavioural assessments conducted by the research team, in the six months prior to the fMRI testing, the patient scored 6-7/23 in the CRS-R, consistent with a VS diagnosis. Similarly, when admitted for fMRI testing, 67 months post injury, the patient scored 7/23, consistent with a diagnosis of VS. (CRS-R subscores: auditory: 1 – auditory startle; visual: 1 – visual startle; motor: 2 – flexion withdrawal; oromotor/verbal: 1 – oral reflexive movement; communication: none; arousal: 2 – eye opening w/o stimulation.) Independent bedside examination, by the attending Neurologist, revealed highly fluctuating and inconsistent signs of awareness that were consistent with a diagnosis of MCS. Nonetheless, despite the best efforts of the research team, it was impossible to establish any signs of awareness or functional communication at the bedside, in any of the CRS-R assessments conducted prior to, or at the time of scanning.

Patient 3. The patient sustained a traumatic brain injury as a result of a motor vehicle accident, in December 1999. He underwent intracranial hematoma and the placement of a ventriculoperitoneal shunt, which was subsequently removed. In the 12 years since the accident, the patient has repeatedly been diagnosed as VS, by the clinical team. In ten behavioural assessments conducted by the research team, in the four months prior to the 1st fMRI testing, the patient scored 4-7/23 in the CRS-R, consistent with a VS diagnosis. When admitted for the 1st MRI testing, 147 months post injury, the patient scored 7/23, consistent with a VS diagnosis. (CRS-R subscores: auditory: 1 – auditory startle; visual: 1 – visual startle; motor: 2 – flexion withdrawal; oromotor/verbal: 1 – oral reflexive movement; communication: none; arousal: 2 – eye opening w/o stimulation.) In the subsequent months, prior to the 2nd fMRI testing, the patient was assessed seven times and maintained a CRS-R score (5-7/23) consistent with a VS diagnosis. Another assessment performed one month after the 2nd fMRI testing, revealed a CRS-R score of 6, again consistent with a VS diagnosis. Throughout the nine-month period since admittance in the research program, additional bedside examinations, by the attending Neurologist, revealed no signs of awareness and were consistent with a VS diagnosis.

Validation of the fMRI Paradigm in Healthy Controls

Prior to patient testing, the fMRI paradigm (eFigure 1) was validated in 15 healthy controls. The paradigm was found to be intuitive and easy to use in untrained participants, and highly accurate for individual participants (90%) (eFigure 2), within rapid response detection times (five minutes). In addition, formal comparison with the current ‘gold-standard’ fMRI method for communication with non-responsive patients revealed improved performance, with respect to individual success rates and amount of scanning time needed to elicit robust responses.
**Basic Sound Perception in Patients**

Prior to testing command-following and communication, we examined each patient’s cortical response during passive sound perception. Sound perception was explored with whole-brain and (independently-defined) region-of-interest analyses, in the case of underpowered contrasts. Sound perception could be explored in each scan, as each contained a silent period, which served as an implicit baseline for the sound trials. Regions-of-interests were defined and tested in different scans. Similarly to healthy controls, significantly more activation for sounds than silence (contrast: ‘Sounds’ > ‘Silence’) was observed in the patients’ left and/or right auditory cortex. Results for each patient are summarized below. Peak coordinates are in each patient’s native space. Patient 1 showed significant activation in auditory cortex, bilaterally (eFigure 3B) ([left: x = –63; y = 25; z = –5; \( P < .001 \), [right: x = 51; y = 10; z = –4; p<0.0001], whole-brain analysis; family-wise error (FWE) cor). Patient 2 showed activation in auditory cortex, bilaterally, which did not reach the statistical threshold (\( P < .001 \), unc) at the whole-brain level (eFigure 3C). Region-of-interest analyses revealed significant/trend activation in the left and right auditory cortex ([left: x = –59; y = –26; z = 5; t = 1.91; \( P < .05 \)], [right: x = 58; y = –37; z = 12; t = 1.45; \( P = .07 \)]). Patient 3 showed significant activation in the right auditory cortex (eFigure 3D) (x = 66; y = –43; z = –18; \( P < .01 \); whole-brain analysis; FWE cor). No significant activation was observed in the left auditory cortex, either at whole-brain or region-of-interest analyses, which is best explained by the widespread atrophy in the patient’s left temporal lobe. In summary, these results confirmed that each patient showed some evidence of basic sound perception.

**Whole-Brain Analyses of Two Communication Scans From Patient 2**

Whole-brain analyses were performed for two of the four communication scans, to explore the patient’s attention response beyond the attention network observed in the command-following scan. Significant effects of attention to one of the words (either ‘yes’ or ‘no’), in either communication scan (B, C), were observed in attention-related regions, with different foci from those observed during the command-following scan (eFigure 5). For example, when asked “Is your name Mike?”, the patient showed significantly more activation for ‘No’ than ‘Yes’ sequences in a cluster subtending the right temporal lobe (eFigure 5B). When asked “Are you in a hospital?”, he showed significantly more activation for ‘Yes’ than ‘No’ sequences in clusters subtending the left middle/inferior temporal and inferior occipital gyri, right parietal, and inferior prefrontal cortex (eFigure 5C). The discrepancy between the activation foci in the command-following (eFigure 5A) and the communication scans (eFigure 5B-C) caused the region-of-interest analysis to fail. However, the direction of significant functional activation in each communication scan was consistent with the correct answer to the question. This suggested that the patient performed the task differently in these two scans as compared to the command-following scan, rather failed to perform.

**References**

The figure displays the design of the two components of the fMRI assessment paradigm. The paradigm was validated in healthy controls prior to patient testing.
eFigure 2. Attention and Communication Scans in 1 Representative Healthy Control

Significant brain activation maps from a representative healthy control in three scanning sessions from the fMRI attention paradigm: (a) command-following; (b-c) communication, question 1; (d-e) communication, question 2. The correct answer to each question (b/c, d/e) (in green) could be decoded from the direction of significant activation (‘Yes’ - ‘No’ contrast), based on the activation pattern from the command-following session (a) (‘Count’ > ‘Relax’ contrast).
Whole-brain results from the contrast ‘Attend’ – ‘Listen’ (i.e., ‘Count’ – ‘Relax’), for (A) the healthy group, and (B-D) each patient are displayed. (A) In the control group, significant brain activity was observed in a network of regions including fronto-parietal, temporal, and pre-/postcentral regions, in the left and right hemispheres (FDR cor.). Results are overlaid on the normalized, group-averaged anatomical volume. (B-D) Significant brain activity (FWE cor.) in patients was observed in a subset of regions activated by healthy controls. Results are overlaid on each patient’s native anatomical volume.
eFigure 4. Basic Sound Perception in Patients

Whole-brain results from the contrast ‘Sounds’ – ‘Silence’, for (A) the healthy group, and (B-D) each patient are displayed. (A) In the control group, significant activation was observed in auditory cortex, including the middle and superior temporal gyri (L, R) (FWE cor.). Results are overlaid on the normalized, group-averaged anatomical volume. (B-D) Each patient showed activation to sounds in the respective auditory cortex regions. Results are overlaid on each patient’s native anatomical volume.
eFigure 5. Whole-Brain Analyses for 2 Communication Scans From Patient 2

Whole-brain analyses of (A) the command-following, and (B-C) two communication scans in Patient 2, clinically diagnosed as minimally conscious. Brain activity is overlaid on the patient’s native anatomical volume. The left and right sides of each panel show significant functional activation ($p<0.05$, FWE corr), in opposite directions of each contrast (i.e., $a > b$, or $b > a$). The direction of significant functional activation indicated the answer (‘yes’ or ‘no’) to each question.
**eTable. Full Statistical Report of Command Following in Patients**

<table>
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<tr>
<th>Patient</th>
<th>Region</th>
<th>Peak Voxel</th>
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<th>P Value</th>
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<td>Patient 3 (eFig. 4D)</td>
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<tr>
<td></td>
<td>Temporal (R)</td>
<td>73 1 32</td>
<td>5.25</td>
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</tbody>
</table>

The voxels correspond to blood-oxygen-level-dependent activity during command-following, shown in eFigure 4B-4D. The coordinates for each patient are given in their native anatomical space. P-values listed are FWE corrected.