● IMAGING IN NEURAL REGENERATION

Neuronal activation by acupuncture at *Yongquan* (KI1) and sham acupoints in patients with disorder of consciousness: a positron emission tomography study

Disorder of consciousness (DOC) is one of the most serious sequelae of brain injury, and is challenging for neurologists and rehabilitation specialists to manage because of its refractory nature (Whyte et al., 2013). Acupuncture is a traditional Chinese medicine technique that is often used to help improve the level of consciousness in patients with DOC. However, the responses to stimulation of acupoints in patients with DOC are not fully understood. It is unclear whether stimulation of acupoints simply provides peripheral sensory input, or whether such stimulation induces specific responses that differ from those of other sensory input. To investigate these responses, we studied five patients with DOC who received acupuncture at real and sham acupoints from January 2012 to June 2013. Positron emission tomography (PET) findings were used to study the effects of the two acupuncture procedures on neuronal activation in the brain.

Patient 1 was a 45-year-old female who had not regained consciousness since an anesthetic accident in January 2009, and was admitted to the China Rehabilitation Research Center in June 2012. She started to open her eyes spontaneously 6 months after the anesthetic accident, but showed no other signs of recovery. Cranial computed tomography (CT) in June 2012 showed diffuse hypoxic brain injury, global atrophy and compensating hydrocephalus. Assessment using the Coma Recovery Scale-Revised (CRS-R) (Kalmar and Giacino, 2005) indicated a vegetative state (Tables 1, 2), and she was admitted for conventional rehabilitation.

Patient 2 was a 47-year-old female who had not regained consciousness since a car accident in August 2011 during which she sustained multiple fractures and internal hemorrhage, and was admitted to the China Rehabilitation Research Center in June 2012. Cranial CT showed diffuse neuronal injury. She was clinically stable after 3 months of treatment, and opened her eyes spontaneously without external stimulation. Assessment using the CRS-R indicated a vegetative state (Tables 1, 2), and she was admitted for conventional rehabilitation.

Patient 3 was a 65-year-old male who had not regained consciousness since the rupture of an intracranial aneurysm. Cranial CT showed intracerebral hemorrhage between the ventricles and blood in the ventricles. He underwent intravascular embolization and ventricular drainage on the day of the rupture. He opened his eyes spontaneously but was unable to communicate. He was admitted to the China Rehabilitation Research Center in June 2012. Assessment using the CRS-R indicated a vegetative state (Tables 1, 2), and he was admitted for conventional rehabilitation.

Patient 4 was a 55-year-old female with a 10-year history of hypertension who had not regained consciousness since an intracranial hemorrhage in July 2011. Cranial CT showed intracerebral hemorrhage in the right thalamus and blood in the ventricles. She underwent decompressive craniectomy and evacuation of the hematoma on the day of the hemorrhage. She started opening her eyes spontaneously 1 month after surgery, but had limited responses to external stimuli. She underwent ventriculoperitoneal shunt placement for hydrocephalus. She was admitted to the China Rehabilitation Research Center in June 2012 for conventional rehabilitation. Assessment using the CRS-R indicated a minimally conscious state (Tables 1, 2).

Patient 5 was a 14-year-old male who had not regained consciousness since accidental ingestion of pesticide in October 2012. He started to open his eyes spontaneously 2 months after the accident, but did not communicate. His level of consciousness gradually improved, and he became more responsive to external stimuli. He was admitted to the China Rehabilitation Research Center in March 2013 for conventional rehabilitation. Assessment using the CRS-R indicated a minimally conscious state (Tables 1, 2).

Intervention after admission: (1) Each patient received acupuncture at bilateral sham acupoints (1 cm lateral to KI 1) during the first scan and at bilateral *Yongquan* acupoints (KI 1, located on the sole of the foot, between the second and third metatarsal bones at the indentation near the front, one-third of the distance from the webs of the toes to the heel) during the second scan, which was performed 3 days after the first scan. (2) All scans were performed at 11:00 a.m., and all patients rested in a dark room for 30 minutes before scanning. CT was performed first, and patients were then injected with 18-fluorodexyglucose (18-FDG) 10 mCi (Chinese PLA General Hospital, synthetized using an Explora FDG4 GE TRACERlab MX FDG Synthesizer accelerator, General Electric Co., Fairfield, Connecticut, USA). PET (Discovery STE scanner, General Electric Co.) was started immediately after injection of 18-FDG. The scan lasted for 50 minutes and dynamic PET data were collected. (3) Acupuncture was performed by an experienced acupuncturist for 30 minutes during the PET scan. The acupuncturist twisted acupuncture needles in alternating clockwise and anticlockwise directions (once per second, ≥ 360°, for 1 minute) every 10 minutes from the start of PET. (4) The following parameters were used for PET: axial field of view: 15 cm; bed: 1; collection mode: 3D reconstruction, 2 iterations, 21 subsets, diameter 2 mm. PET data were reconstructed with slice thickness 3.73 mm, slice interval 1 mm, and matrix size 128 × 128. (5) PET-CT images were processed using Statistical Parametric Mapping software (SPM2; The Welcome Department of Cognitive Neurology, University College London, UK). After realignment, the images were normalized using the Montreal Neurological Institute template and then smoothed using a Gaussian kernel with 6 mm full width at half-maximum. The half-width of the X, Y and Z axes was 10 mm. Spatial data were determined using SPM2 software, and differences between the two acupuncture procedures were analyzed using the paired t-test. (6) Differences between the two acupuncture procedures were analyzed using the t-test to acquire the t and Z values of corresponding pixel points, and a statistical parameter map was constructed based on t/Z values. The threshold for statistical significance was set at $P < 0.05$ with correction for false-discovery rate, and the cluster size threshold was set at > 5 voxels.

For $P = 0.001$ ($t = 4.303$) and extent threshold ≤ 300, there was significantly higher metabolism in the left putamen, left anterior cingulate cortex, left gyri orbitales, bilateral cerebellar hemispheres and right paracentral lobule during stimulation of the *Yongquan* acupoints than the sham acupoints (Table 3, Figure 1).

Neuronal activity can be observed on PET after injection of ^{18}F-FDG, which shows areas of synaptic firing in the brain (Hsieh et al., 2011; Eidelberg et al., 1997). The results of this study show that acupuncture at the *Yongquan* acupoints induced stronger neuronal activity than acupuncture at the sham acupoints. We believe that acupuncture at the *Yongquan* acupoints may increase synaptic activity in some areas of the brain. The putamen (Palmiter, 2011), cingulate cortex, frontal lobe (Boly et al., 2013) and cerebellum (Sullivan, 2010) are involved in conscious thought. Long-term acupuncture may affect the quantity and function of synapses in these areas, leading to neural reorganization. This may explain the mechanism by which acupuncture at the *Yongquan* acupoints results in improvement of patients with DOC.

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Table 1 Baseline data of five patients

Case	Sex	Age (year)	H (cm)	W (kg)	Pathogeny	Onset time	Course of disease (month)	Admission time	Type of DOC	Location of lesion	Diagnosis at onset time	Treatment	Diagnosis at admission	Treatment after admission
1	F	45	163	57	Anesthetic accident	January 2009	43	January 2012	VS	Diffusive	Anesthetic accident	Symptomatic treatment	Diffusive hypoxic encephalapathy; global dncephalatrophy; hydroencephalus	Conventional rehabilitation
2	F	46	165	50	Traumatic brain injury	August 2011	6	June 2012	VS	Diffusive	Traumatic brain injury	Surgery	Chronic traumatic brain injury	Conventional rehabilitation
3	M	65	174	65	Intracranial hemorrhage	January 2012	5	June 2012	VS	Between lateral ventricles	Intracranial hemorrhage	Surgery	Chronic intracranial hemorrhage	Chronic intracranial hemorrhage
4	F	55	165	40	Intracranial hemorrhage	July 2011	11	June 2012	MCS	Bilateral cerebral hemisphere	Intracranial hemorrhage	Surgery	Chronic intracranial hemorrhage	Chroni intracranial hemorrhage
5	M	14	172	56	Pesticide toxication	October 2012	7	May 2013	MCS	Diffusive	Pesticide toxication	Symptomatic treatment	Toxic encephalopathy	Conventional rehabilitation

Vegetative state (VS) refers to the neurocognitive status of individuals with severe brain damage, in whom physiologic functions (sleep-wake cycles, autonomic control, and breathing) persist, but awareness (including all cognitive function and emotion) is abolished. Minimally conscious state (MCS) is a type of disorder of consciousness (DOC) where partial consciousness is preserved, which differs from coma and VS. MCS can develop in patients with coma or VS resulting from acute brain injury, or with congenital diseases of the nervous system. F: Female; M: male; H: height; W: weight.

Table 2 CRS-R assessment of the five patients at admission

Subset of CRS-R	Case 1	Case 2	Case 3	Case 4	Case 5
Hearing	1	0	0	2	0
Vision	0	1	1	3	3
Motor	2	2	2	2	2
Response to speech	2	1	0	1	2
Communication	0	0	0	0	0
Wakefulness	2	2	2	2	2

For diagnosis of a vegetative state according to the CRS-R criteria, all of the following are required: hearing ≤ 2, vision ≤ 1, motor ≤ 2, speech ≤ 2, communication = 0, wakefulness ≤ 2. For diagnosis of a minimally conscious state, any one of the following is required: hearing = 3–4, vision = 2–5, motor = 3–5, speech = 3, communication = 1. For diagnosis of recovery from a minimally conscious state, either one of the following is required: motor = 6, communication = 2. CRS-R: Coma Recovery Scale-Revised.

Table 3 Brain areas activated by acupuncture in patients with disorder of consciousness: sham acupoints versus *Yongquan* (KI1) acupoints

Activated anatomic area	Side	*t* value (paired *t*-test) peak three-dimensional coordinate				
		X	*Y*	*Z*	*t*	*P*
Cingulate gyrus	Left	–2	16	34	4.16	0.001
Putaman	Left	–14	6	–16	3.96	0.009
Gyri orbitales	Left	–10	48	–16	3.65	0.010
Cerebellum	Left	–16	–40	–24	3.65	0.028
Cerebellum	Right	12	–68	–46	3.64	0.010
Paracentral lobule	Right	26	–46	54	3.47	0.030

All results are thresholded at a whole-brain false-discovery rate corrected $P < 0.05$. Metabolism was significantly higher in the left putamen, left anterior cingulate cortex, left gyri orbitales, bilateral cerebellar hemispheres and right paracentral lobule at the time of acupuncture at *Yongquan* (KI1) acupoint (paired *t*-test; $P < 0.05$).

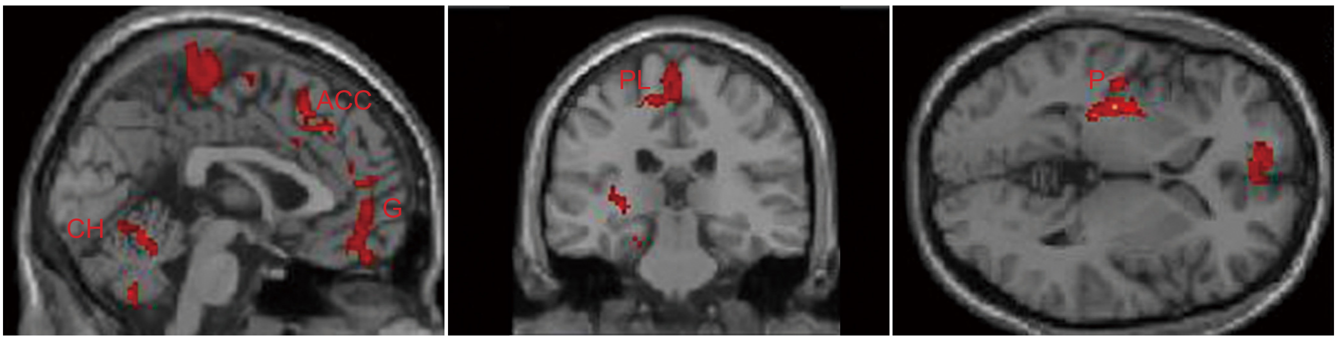

Figure 1 Brain activation during acupuncture at the *Yongquan* acupoint (KI1) in five patients.
The figure shows the statistical parameter map generated using SPM2 software, comparing the PET-CT data showing areas of activation during acupuncture at the sham and *Yongquan* acupoints. Left: Sagittal section; middle: coronal section; right: transverse section. The red color indicates areas of activation. P: Putamen; G: gyri orbitales; PL: paracentral lobule; ACC: anterior cingulate cortex; CH: cerebellum hemisphere.