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The integrated response of the human cerebro-cerebellar and limbic systems to acupuncture stimulation at ST 36 as evidenced by fMRI

Kathleen K.S. Hui,[a,*] Jing Liu,[a] Ovidiu Marina,[a] Vitaly Napadow,[a] Christian Haselgrove,[b] Kenneth K. Kwong,[a] David N. Kennedy,[b] and Nikos Makris[b]

[a]*Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Building 149, 13th Street, Room 2301, Charlestown, MA 02129, USA*
[b]*Center for Morphometric Analysis, Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA 02129, USA*

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Clinical and experimental data indicate that most acupuncture clinical results are mediated by the central nervous system, but the specific effects of acupuncture on the human brain remain unclear. Even less is known about its effects on the cerebellum. This fMRI study demonstrated that manual acupuncture at ST 36 (Stomach 36, *Zusanli*), a main acupoint on the leg, modulated neural activity at multiple levels of the cerebro-cerebellar and limbic systems. The pattern of hemodynamic response depended on the psychophysical response to needle manipulation. Acupuncture stimulation typically elicited a composite of sensations termed *deqi* that is related to clinical efficacy according to traditional Chinese medicine. The limbic and paralimbic structures of cortical and subcortical regions in the telencephalon, diencephalon, brainstem and cerebellum demonstrated a concerted attenuation of signal intensity when the subjects experienced *deqi*. When *deqi* was mixed with sharp pain, the hemodynamic response was mixed, showing a predominance of signal increases instead. Tactile stimulation as control also elicited a predominance of signal increase in a subset of these regions. The study provides preliminary evidence for an integrated response of the human cerebro-cerebellar and limbic systems to acupuncture stimulation at ST 36 that correlates with the psychophysical response.

Keywords: Acupuncture; Negative BOLD; Limbic; Cerebrum; Cerebellum; Sensations; Pain; Affect; Cognition

Introduction

Acupuncture, an ancient healing technique that originated in China, is gaining popularity in the Western medical community for the treatment of diverse disorders (NIH, 1998). Traditional acupuncture involves stimulation with very fine needles inserted into defined sites on the body, eliciting a composite of sensations, termed *deqi*, which is considered to be related to clinical efficacy in traditional Chinese medicine (Cheng, 1997; Helms, 1995; Vincent et al., 1989). In clinical practice, different acupuncture points can be used to treat the same disorder, whereas one acupuncture point can be used to treat different disorders (Cheng, 1997; Stux and Hammerschlag, 2001). A body of clinical and experimental evidence indicates that most acupuncture effects are mediated via the brain (Han et al., 1982; Stux and Hammerschlag, 2001). However, the central effects of acupuncture and the neural correlates of *deqi* remain unclear.

Modern neuroimaging has provided revolutionary tools to monitor the dynamic response of the whole brain to acupuncture with specific regional localization. Functional MRI and PET studies on acupuncture at commonly used acupuncture points have demonstrated significant modulatory effects on the limbic system, paralimbic and subcortical gray structures (Hsieh et al., 2001; Hui et al., 1997, 2000; Kong et al., 2002; Napadow et al., 2005; Wu et al., 1999, 2002; Zhang et al., 2003). The role of the cerebellum in autonomic control, cognition and affect, as well as in sensorimotor control is gaining increasing attention (Schmahmann, 1991, 2000a; Schmahmann and Sherman, 1998). Clinical and experimental data indicate that direct and indirect links connect the cerebellar vermis and deep nuclei with subcortical and cortical limbic/paralimbic regions in the cerebrum and brainstem, including the amygdala, hippocampus, septal nuclei, cingulate gyrus, hypothalamus, nucleus accumbens, prefrontal cortex, ventral tegmental area and reticular formation (Heath et al., 1978; Helmchen et al., 2003; Kelly and Strick, 2003; Leiner et al., 1989; Leroi et al., 2002; Paradiso et al., 1999; Price, 2000; Schmahmann and Pandya, 1997). Investigators are beginning to explore the effects of acupuncture on the cerebellum by fMRI (Hui et al., 2003; Yoo et al., 2004). Now that methods for specific

* Corresponding author. Fax: +1 617 726 7422.
E-mail address: hui@nmr.mgh.harvard.edu (K.K.S. Hui).
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regional localizations of the cerebellum have become available (Makris et al., 2003, 2005), whole brain imaging provides the opportunity to explore the functional relationships and connectivity between multiple levels of the human brain.

We hypothesize that the multifaceted effects of acupuncture performed at main classical acupuncture points involve brain circuits that can regulate and integrate diverse somatic and mental functions in a coordinated manner, and that the limbic system may play a central role. We further postulate that the limbic and paralimbic structures in the cerebro-cerebellar system respond in a concerted manner to acupuncture stimulation, and that the central effects as evidenced by fMRI and psychophysical response are interrelated. Our previous fMRI data collected during acupuncture at classical acupuncture points, LI 4 (large intestine 4 or *Hegu*) on the hand and ST 36 (stomach 36 or *Zusanli*) on the lower leg, demonstrated a predominance of signal attenuation, particularly in the limbic/paralimbic regions of the telencephalon, diencephalon and brainstem (Hui et al., 1997, 2000, 2003; Napadow et al., 2005). In this study acupuncture was performed at ST 36, a main acupoint that is used for treating a variety of medical conditions. The cerebellum was examined for region-specific and quantified effects of acupuncture, using the new parcellation method introduced by one of the authors (Makris et al., 2003, 2005). The results demonstrated an integrated response of the human cerebro-cerebellar and limbic systems.

Materials and methods

Participants

The study was performed on 15 right-handed, acupuncture-naïve healthy adult volunteers with informed consent, as approved by the Massachusetts General Hospital Subcommittee on Human Studies. Subjects were screened and excluded for neurological, mental and medical disorders, intake of conventional or alternative medicine, head trauma with loss of consciousness and contra-indications for exposure to high magnetic fields. Eight male and seven female subjects, 22–47 years old (29.8 ± 7.5 years), 1 Asian, 2 Hispanic and 12 white, participated in the study. They were acupuncture naïve and were limited to participation in a single session of the study.

Experimental paradigm

The investigation was conducted at the Athinoula A. Martinos Center for Biomedical Imaging. The present report is derived from data of a larger study that required approximately 2 h for data acquisition. Anatomical scans of the brain and functional images of sensory control stimulation were collected prior to acupuncture imaging. The subjects were told acupuncture was to be performed at different sites with different techniques that would generate different sensations during the needling. They were not informed of the order in which the sensory and the acupuncture stimulations would be performed. They were instructed to lie still and keep their eyes closed during the 10-min scan. At the end of each 10-min scan, the subjects were questioned about the sensations that they had felt during the stimulation and whether they were anxious or relaxed during the procedure. They could not see their lower extremities from their supine position in the enclosed scanner; and being acupuncture-naïve, they would be unable to discriminate the tactile stimulation control from real acupuncture until they experienced real acupuncture. Acupuncture was performed using sterile disposable stainless steel needles at three acupuncture points on the right extremity in separate runs: ST 36 on the leg, LI 4 on the hand and LV 3 (Liver 3 or *Taichong*) on the foot. The order of the acupoints used for stimulation was randomized. Only the results of ST 36 from this cohort of subjects will be presented in this report. Analysis on data from other acupoints is in progress.

The acupuncture point ST 36 is located in the tibialis anterior muscle, 4 fingerbreadths below the kneecap and 1 fingerbreadth lateral from the anterior crest of the tibia. Disposable sterile stainless steel needles (KINGLI Medical Appliance Co., Ltd., Wuxi, China) of 0.22 mm in diameter and 40 mm in length were used. The needle was inserted vertically to a depth of 2–3 cm. The sensitivity of the subject to needle manipulation was tested and adjusted to tolerance prior to scanning. In the event of a sharp painful sensation, the needle position would be readjusted and the pain would disappear within a few seconds. Stimulation consisted of rotating the needle bidirectionally with an even motion to an amplitude of approximately 180° at the rate of one cycle per second. This approximates a technique used in clinical practice. The total scanning time was 10 min per run. The needle was kept in place for 2 min prior to needle manipulation. The two stimulation blocks S1 and S2 were separated by an interval of 3 min with needle kept in place. Scanning continued for 1 min after S2 (Fig. 1). Duplicate runs were performed at each acupuncture point.

Sensory control stimulation consisted of tapping the skin gently at the acupuncture point with a size 5.88 von Frey monofilament using a paradigm matched to that of the acupuncture runs for the subject. Due to time limitation, only one of the 3 acupoints to be compared could be studied on a single subject in one session.

Imaging

Brain imaging was conducted on a 1.5-T Siemens Sonata MRI system equipped for echo planar imaging (EPI) with a standard head coil. Functional scans were collected with sagittal sections parallel to the AC-PC plane, slice thickness 3.0 mm with 20% gap. Imaging encompassed the entire brain, including the cerebellum and brainstem. The functional data were acquired by a T2*-

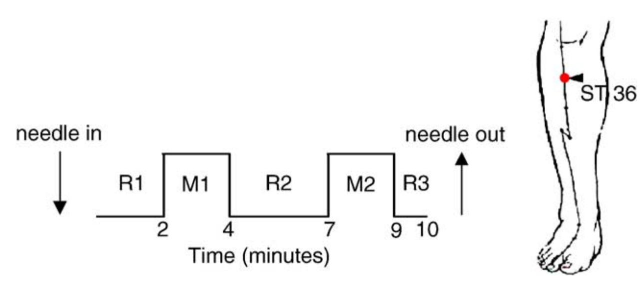

Fig. 1. Acupuncture was performed at acupuncture point ST 36 on the right leg (arrowhead). The acupuncture needle was inserted and the sensitivity of the subject to manipulation was pre-tested and adjusted to tolerance prior to each scanning run. After remaining at rest for 2 min, the needle was rotated bidirectionally with even motion at the rate of 1 Hz for 2 min. After a rest period of 3 min, needle manipulation was repeated in like manner. The needle was removed at the end of the 10-min experimental run. The signal intensities with the needle at rest before, between and after manipulation served as the baseline for comparison with the signal intensities during manipulation.

weighted gradient echo sequence (TE 30 ms, TR 4 s, matrix 64 × 64, FOV 200 mm, flip angle 90°, in-plane resolution 3.125 × 3.125 mm). A set of 3D MPRAGE (magnetization-prepared rapid acquisition gradient echo) images, voxel size 1 mm^3, 128 images per set, and a set of T1-weighted high-resolution structural images (TE 3.39 ms, TR 2.73 s, matrix 192 × 256, FOV 256 mm, flip angle 7°, in-plane resolution 1 × 1 mm, slice thickness 1.33 mm) were acquired prior to functional scans.

Psychophysical sensations

At the end of the 10-min scan, the subject was questioned whether aching, pressure, soreness, heaviness, fullness, warmth, coolness, numbness, tingling, dull pain, sharp pain or other sensations had occurred during the stimulations, and, if present, to rate the intensity of each sensation on a scale of 1 to 10 (0 = no sensation, 1–3 = mild, 4–6 = moderate, 7–8 = strong, 9 = severe and 10 = unbearable sensation). In order to minimize bias, the subject was told prior to the test procedures that the type and intensity of sensations would vary with different individuals and different acupoints, and that a range from nil to multiple sensations could be experienced for any given run. Although the subject was to refrain from motion and to remain relaxed during the scan, they were asked to signal to the acupuncturist by raising one finger if *deqi* reached a score of 8 and raising 2 fingers in the event of sharp pain. When so signaled, the acupuncturist would adjust the intensity of stimulation by reducing the angle of rotation of the needle. In general, the undue discomfort would disappear almost immediately, well within the TR interval of 4 s.

Psychophysical data analysis

Deqi, or needling sensation, is the constellation of sensations a person feels during acupuncture needle manipulation, such as aching, pressure, soreness, fullness, distension, numbness, tingling, local warm or cool sensations, pain and the spreading of these sensations (Cheng, 1997; Park et al., 2002; Pomeranz, 1991; Vincent et al., 1989). The needling technique adopted in this study was gentle; the aim was to generate *deqi* with little or no sharp pain. Whereas dull pain was considered as a component of *deqi*, sharp pain was considered as inadvertent noxious stimulation. The data sets were categorized for analysis according to the sensations reported as follows: (1) *deqi* and (2) *deqi* plus sharp pain (mixed sensations). In this cohort, none of the subjects experienced sharp pain without *deqi*.

fMRI data analysis

The signal intensities with the needle at rest before, between and after the needle manipulation periods served as the baseline for the assessment of changes in signal intensity induced by needle manipulation. Images were processed using the AFNI software program (Cox, 1996). The data were first motion corrected; data runs were excluded if gross motion exceeded 2 mm on any axis. Statistical parametric mapping was completed via a generalized linear model. The estimated response function was then compared by a *t* test to the time series data in each brain voxel (3dDeconvolve, AFNI). The spatial extent of our EPI images was checked to ensure that the data from susceptibility-sensitive regions were not corrupted by susceptibility-induced signal drop-off. The statistics were color-coded and mapped onto the subject's own high-resolution 3D anatomical data set in Talairach space (Talairach and Tournoux, 1988). No spatial or temporal pre-processing smoothing was done on the data.

In individual analysis, data from duplicate runs for acupuncture or for sensory control at an acupuncture point, if available, were averaged. The data were then transformed into Talairach space, normalized to average image intensity and blurred with a spatial Gaussian filter (full-width half-max of 2 mm) to compensate for any residual differences. The level of significance was thresholded at $P < 0.003$ ($t > 3.02$) and a minimum cluster size of 3 voxels. The time course of signal change was visually compared with the experimental paradigm. Signal changes that failed to agree with the paradigm were rejected as artifacts. The percent of signal change was taken from the voxel with maximal change for each structure. When signal increases and signal decreases coexisted in the same structure, the predominant pattern was selected. In the absence of predominance of any one pattern, both were listed. In order to address the multiple comparison correction, a Monte Carlo simulation was completed, the results of which demonstrated that our combination of clustering and thresholding produced a false-positive discovery rate, α, of less than 0.6% (AlphaSim, AFNI).

In group analysis, group-averaged functional statistical maps were registered onto the group-averaged high-resolution anatomical maps of the subjects. Statistical significance was thresholded at $P < 0.001$ ($t > 3.37$) and a minimum cluster size of 3 voxels, with α less than 0.4% (AlphaSim, AFNI).

The anatomical definitions of the regions of interest in the cerebral circuits were based on methods previously published through the Center for Morphometric Analysis, Department of Neurology, MGH (Caviness et al., 1996; Filipek et al., 1994). These have been applied and adapted in a number of studies (Breiter et al., 1997; Hui et al., 2000). The anatomical localization and labeling of the functional data were determined by both Talairach coordinates and direct inspection based on these definitions.

The hemodynamic response of the cerebellum was derived together with the response of the rest of the brain using AFNI for data analysis as previously described (Napadow et al., 2005). The AFNI data were imported into FreeSurfer, and the functional activations were mapped onto the exterior surface of the cerebellum that was reconstructed from the anatomical images (Dale et al., 1999; Fischl et al., 1999). Only signal changes that were located at or were contiguous with the exterior surface of the cerebellum were projected onto the cerebellar surface, as shown in Fig. 8. Signal changes located at the interface between white and gray matter that did not reach the exterior surface were not included. The regions of interest in the cerebellum were localized using cross sections in 3D Talairach space and by the surface-assisted cortical parcellation method (Makris et al., 2003, 2005). The anatomical definitions of the regions of interest were based on this parcellation method and on the MRI Atlas of the Human Cerebellum (Schmahmann et al., 2000).

Results

Psychophysical response

Among the 15 subjects in the study, 11 experienced *deqi* (designated as *deqi*) and 4 experienced *deqi* mixed with sharp

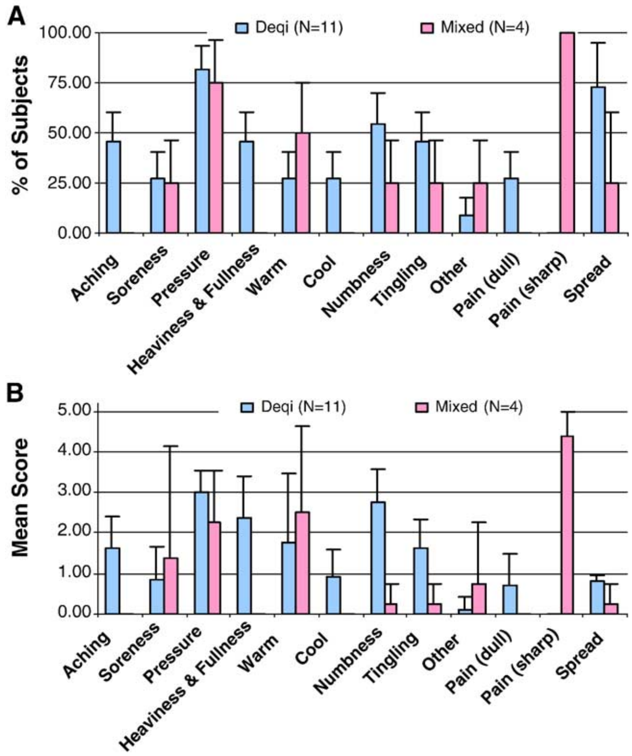

Fig. 2. Subjects were categorized based on whether they reported the sensations listed for *deqi* but not sharp pain (*deqi*), or whether they reported sharp pain as well as *deqi* sensations (*mixed sensations*). (A) The percentage of subjects who reported having felt the given sensation, with binomial error bars. The most frequently reported sensation was pressure, followed by aching and numbness. (B) The average score, on a scale from 0 denoting no sensation to 10 denoting an unbearable sensation, for each sensation, with standard error bars. Spreading of any sensation was noted in a binary fashion and coded as follows: 1—spreading reported; 0—spreading not reported.

pain (designated as mixed sensations). In subjects with mixed sensations, *deqi* remained predominant. The prevalence of these sensations is expressed as the percentage of individuals in the group that reported the given sensation (Fig. 2A). The intensity is expressed as the average score ± SE (Fig. 2B). For the *deqi* group, the sensations in the order of decreasing frequency were as follows: pressure (82%), aching, heaviness, fullness, numbness and tingling (45–55%), dull pain, soreness, and warm or cool feeling around the needling site (27%). With mixed sensations, pressure also led the list (75%), followed by warm sensation (50%), and soreness, numbness and tingling. Numbness and tingling tended to be less common and weaker in mixed sensations than in *deqi* (scores of 0.25 vs. 2.75 for numbness and 0.25 vs. 1.63 for tingling). The sharp pain was of moderate intensity, lasting at most a few seconds, and not more than 3 times during each 2-min needle manipulation period. On the other hand, the dull pain associated with *deqi* often persisted throughout the needling manipulation and frequently occurred in the absence of sharp pain. Importantly, dull pain did not activate the structures related to noxious stimulation but caused deactivation as seen with *deqi.* Interview records showed that the subjects remained relaxed during the procedures, except when a procedure inadvertently produced sharp pain. They adapted well to the novel environment although it was not relaxing as in acupuncture clinic settings.

Hemodynamic response

Acupuncture stimulation produced distinct patterns of hemodynamic response that differed between different categories of psychophysical response. As illustrated in parasagittal sections in Fig. 3, extensive signal decreases occurred in the cerebrum, brainstem and cerebellum in the *deqi* group. When *deqi* was mixed with pain, the hemodynamic response also became mixed, with signal increase becoming the predominant pattern. In the sensory control, signal changes were sparse. The signal change was also predominantly positive, except for the ventro-medial prefrontal cortex that showed focal negative activation. Interestingly, the secondary somatosensory cortex (SII) demonstrated stronger activation in tactile stimulation than in acupuncture needle stimulation with *deqi* (Fig. 4 and Table 1).

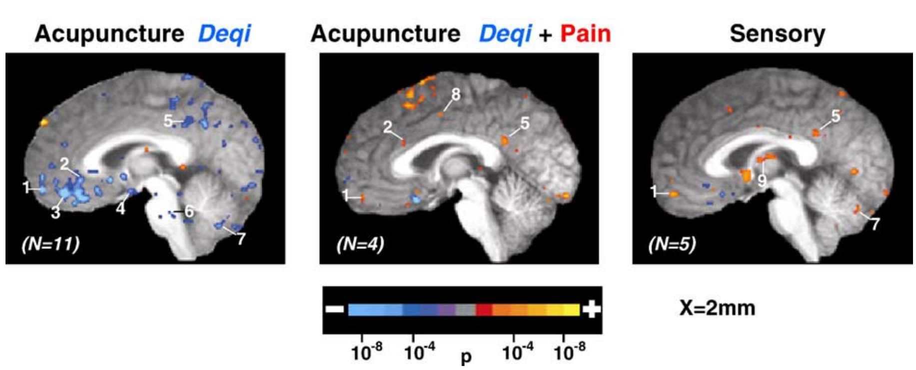

Fig. 3. The influence of subjective sensations on fMRI signal changes of the brain during acupuncture and sensory control performed at ST 36. Group average functional results were thresholded at $P < 0.001$, cluster size of at least 3 voxels. All slices were taken sagittally at 2 mm to the right of the midline in Talairach space. Functional statistical maps (P values) were overlaid on the respective average anatomical scans. Blue denotes signal decreases, and yellow to red signal increases. Regions: 1—frontal pole; 2—subgenual anterior cingulate, Brodmann area 24; 3—ventromedial prefrontal (VMPF) cortex; 4—hypothalamus; 5—posterior cingulate; 6—reticular formation; 7—cerebellar vermis (detailed in Fig. 6); 8—middle cingulate, Brodmann area 32; and 9—thalamus. (Left) Acupuncture with *deqi* sensations (N = 11) but without sharp pain resulted in widespread signal decreases, including the frontal pole, VMPF cortex, cingulate cortex, hypothalamus, reticular formation and the cerebellar vermis. (Center) Acupuncture with *deqi* and sharp pain sensations (N = 4) resulted in signal increases in several areas, including the frontal pole and the anterior, middle and posterior cingulate. (Right) Sensory control (N = 5) resulted in signal increases in the frontal pole, posterior cingulate, thalamus and cerebellar vermis.

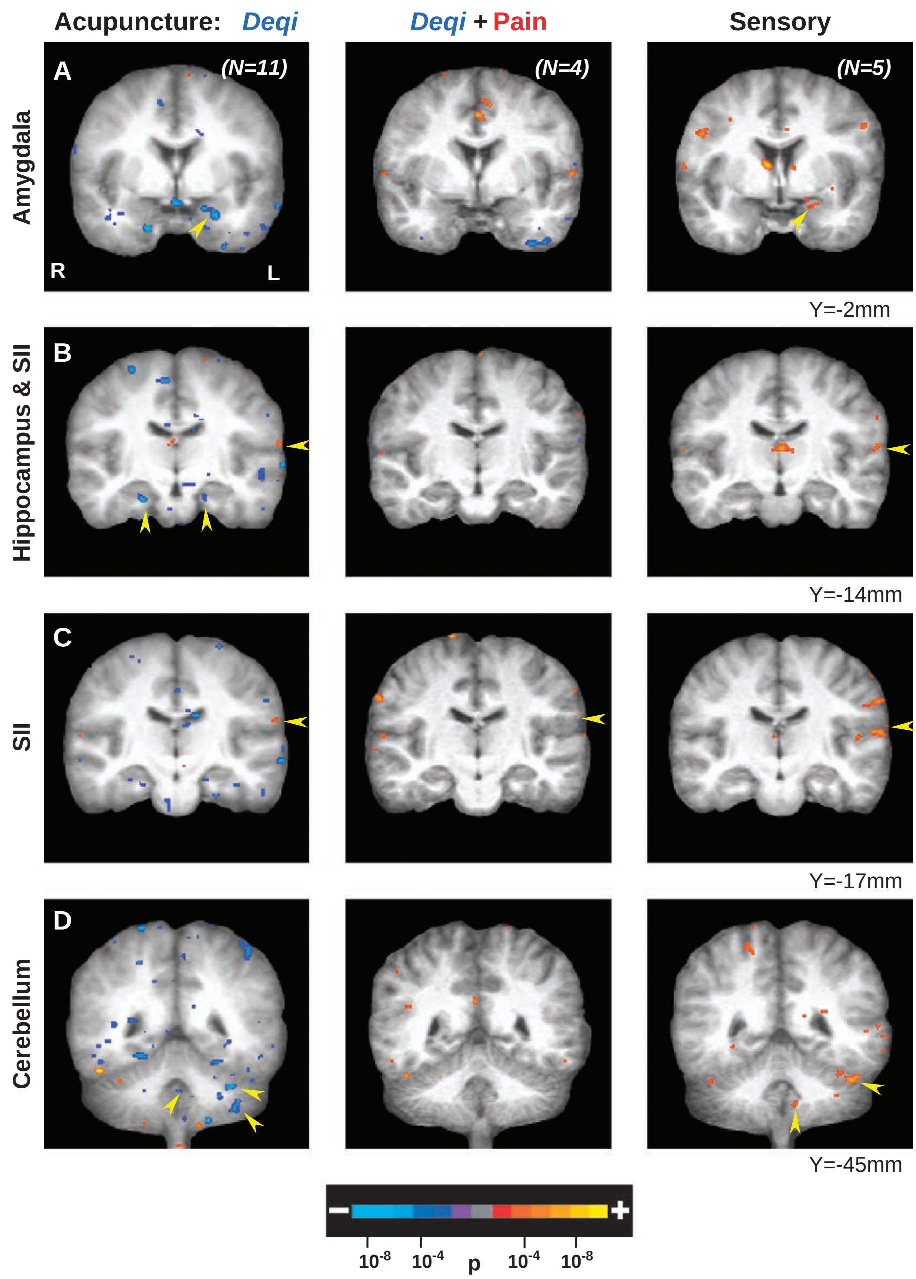

Fig. 4. The influence of subjective sensations on fMRI signal changes on major limbic structures, the secondary somatosensory cortex (SII) and the cerebellum during acupuncture at ST 36. The coronal slices were taken through the same data sets presented in Fig. 3 using the same criteria. Regions of interest are denoted by yellow arrowheads. (Left) Acupuncture with *deqi* sensations ($N = 11$). (Middle) Acupuncture with mixed sensations of *deqi* and sharp pain ($N = 4$). (Right) Sensory control ($N = 5$). Row A: The amygdala showed signal decrease with acupuncture *deqi*, increase with sensory stimulation and no significant change with acupuncture mixed sensations. (Row B) The hippocampus, bottom arrows, showed signal decrease with acupuncture *deqi*, and no significant change otherwise. (Row C) SII, also shown by the right arrows in Row B, shows signal increase under all three stimulations. Acupuncture, being a form of sensory stimulation, would be expected to result in signal increases in SII, which is in stark contrast to the widespread signal decreases during acupuncture *deqi*. (Row D) With acupuncture *deqi*, the cerebellum showed signal decreases in the vermis and lobules VI and VII. With sensory control, the lateral hemisphere showed signal increases. Acupuncture with mixed sensations resulted in no significant hemodynamic response in this section of the cerebellum, but signal changes, with increases predominating, appeared in other sections (see Table 2).

Acupuncture: deqi (N = 11)

Acupuncture with *deqi* resulted in a marked predominance of signal attenuation or deactivation in the cerebro-cerebellar and limbic systems (Tables 1 and 2; Figs. 3–7). The magnitude of signal change was small, generally less than 1%, much less than the change elicited by pain or other sensory tasks reported in the literature. In the cerebellum the changes generally ranged from 0.2% to 0.7% in most regions (Table 2, Fig. 5). The effect was most extensive in the posterior vermis, involving lobules VI through X. In the anterior vermis, signal attenuation was limited to lobules III and V (Table 2A; Figs. 3–7). In the medial hemispheres, prominent changes, exceeding 1%, in some regions, were observed in VIIA Crus I, VIIB and VIIIB (Table 2B; Fig. 5, middle

Table 1
FMRI signal changes in the cerebrum and brainstem – *Deqi* vs. *Deqi* + pain

			Acupuncture										Sensory				
			Deqi					*Deqi* + **pain**									
			N = 11					N = 4					N = 5				
		BA	Talairach			Δ	P	Talairach			Δ	P	Talairach			Δ	P
			x	y	z	signal	value	x	y	z	signal	value	x	y	z	signal	value
A. Limbic and paralimbic			(mm)			(%)	(log)	(mm)			(%)	(log)	(mm)			(%)	(log)
Amygdala	R		25	-7	-18	-0.18	-3.43						14	10	-7	0.49	-4.36
	L		-24	-2	-19	-0.39	-7.33						-19	-5	-11	0.34	-3.99
Hippocampus	R		34	-22	-9	-0.24	-4.93										
	L		-17	-16	-16	-0.48	-5.41										
Parahippocampus	R		17	-2	-28	-0.88	-10.50	25	-19	-24	0.96	-4.95					
	L		-26	-7	-29	-0.58	-7.94										
Hypothalamus	R		2	-3	10	-0.43	-6.73										
	L		-2	-4	12	-0.91	-9.26										
Septal area	R		2	1	10	-0.28	-4.27										
	L		-2	1	10	-0.34	-7.01										
Nucleus accumbens /	R		8	7	-6	-0.42	-5.98										
SCC	L		-5	9	-1	-0.25	3.90										
Anterior cingulate –	R	32	4	31	-6	-0.63	-8.23						3	30	-6	-0.37	-4.69
subgenual	L	24	-1	29	-2	-0.61	-7.79										
Anterior cingulate –	R	24	1	33	2	-0.48	-5.74										
pregenual	L	24	-6	34	9	-0.31	-4.94						-2	32	0	0.40	-3.59
Anterior cingulate –	R	24						1	22	23	0.44	-4.80					
dorso-anterior	R	32	2	41	21	-0.30	-6.26										
	L	24															
Anterior middle cingulate	R	24						1	7	32	0.43	-3.98					
	R	32															
	L	24						-2	-8	31	0.58	-5.90	-2	5	3	0.39	-3.71
	L	32															
Posterior middle	R	24	2	-16	37	-0.20	-3.87	1	-2	41	0.56	-7.04	2	-3	33	0.43	-5.39
cingulate	L	24	-2	-16	37	-0.25	-4.65	-2	-2	40	0.53	-6.10					
Posterior cingulate	R	23	5	-40	34	-0.28	-4.61	3	-44	23	0.78	-5.60	2	-25	25	0.38	-4.96
	L	23	-2	-37	33	-0.29	-5.05	-1	-41	22	0.76	-5.63	-3	-40	31	0.48	-8.06
Retrosplenial cortex	R	31	3	-42	37	-0.30	-6.16										
	R	30	7	-51	14	-0.44	-7.83						4	-60	19	0.36	-4.23
	L	30	-5	-57	19	-0.33	-5.74						-3	-48	10	0.66	-4.53
	L	30	-5	-52	20	-0.37	-6.85										
Anterior insula	R												34	18	3	0.29	-4.59
	L		-39	8	-6	-0.32	-4.32						-32	14	8	0.34	-6.36
Posterior insula	R												49	13	-7	0.63	-7.85
	L																
Temporal pole	R		25	14	-25	-0.74	-8.48	45	13	-10	0.80	-5.66	49	13	-7	0.63	-7.85
	R							34	12	-30	-0.62	-6.88					
	L		-26	11	-29	-0.45	-7.64	-35	2	-36	-1.25	-8.64	-52	10	-4	0.68	-4.93
Frontal pole	R	10	2	65	-10	-1.04	-11.47	8	59	26	0.88	-7.53	4	64	1	1.00	-9.06
	R	10						8	62	8	-1.05	-7.48					
	L	10	-21	53	7	-0.40	-6.84	-5	59	20	0.95	-8.20	-2	61	11	0.75	-7.18
	L	10						-5	59	8	-0.50	-4.09	-10	61	0	-0.76	-8.90
VMPF cortex –	R		4	31	-14	-1.00	-13.06	2	14	-16	-2.22	-15.04	5	17	-13	-0.65	-4.77
BA 11 & 12	L		-3	39	-10	-0.76	-9.60	-4	22	-18	1.49	-7.28	-1	27	-12	0.44	-6.11
VMPF cortex –	R	25	3	17	-13	-0.86	-6.75						3	12	-11	-0.95	-3.73
BA 25	L	25	-2	5	-7	-0.34	-3.61										
B. Subcortical structures																	
Caudate	R		14	13	8	-0.27	-7.16						-9	-12	7	0.45	-4.44
	L		-7	13	8	-0.35	-7.86						-8	12	8	0.33	-3.44
Putamen	R							20	4	5	0.49	-3.98	19	12	-2	0.47	-7.19
	L												-22	8	2	0.36	-4.77

Table 1 (*continued*)

Anterior thalamus	R															
	L											-8	-10	17	0.33	-3.51
Middle medial thalamus	R											6	-13	10	0.34	-3.93
	L											-6	-13	11	0.38	-5.74
Posterior thalamus	R	16	-29	3	-0.30	-5.45										
	L	-10	-31	5	-0.28	-4.52						-11	-26	11	0.27	-3.50
C. Sensorimotor and association cortex																
Paracentral lobule	R	4	-47	60	0.27	-3.51										
	L	-7	-36	59	0.32	-4.06										
SII	R	60	-23	27	0.34	-4.48	56	-25	23	0.61	-8.14	55	-25	22	0.71	-13.00
	L	-62	-14	14	0.49	-5.79	-59	-20	21	0.72	-7.40	-55	-22	29	0.35	-4.20
Inferior parietal lobe	R	56	-35	35	-0.19	-3.62	48	-44	40	0.41	-3.94	53	-36	41	0.42	-5.76
	R						53	-57	35	-0.62	-4.01					
	L	-37	-41	47	-0.24	-3.57						-47	-35	25	0.46	-8.15
Premotor cortex	R	4	-14	54	-0.33	-6.54	3	2	60	0.48	-3.77	39	4	48	0.37	-4.17
	L	-4	-19	63	-0.37	-6.28	-4	-10	63	0.51	-5.06					
D. Brainstem																
Ventral tegmental area / substantia nigra	R	11	-19	-10	-0.31	-4.04										
	L	-4	-20	-15	-0.33	-5.40										
Reticular formation	R	4	-31	-27	-0.31	-6.10										
	L	-5	-28	-31	-0.28	-3.55										
Pontine nuclei	R	5	-17	-28	-0.47	-5.06										
	L	-1	-22	-28	-0.27	-4.99										
Dorsal raphe	R	2	-23	-7	0.20	-3.63										
	L	-2	-22	-6	0.28	-5.51										

The influence of subjective sensations on fMRI signal changes in the cerebrum and brainstem during acupuncture and sensory control at right. ST 36: The voxel with the maximal signal change was selected for each region. Group data were thresholded at $P < 0.001$ with a cluster of at least 3 voxels. Values for regions showing signal decreases are shown in blue, and increases in red. Acupuncture with *deqi* ($N = 11$) elicited a predominant pattern of signal attenuation in both cerebrum and brainstem, in stark contrast to signal enhancement when *deqi* was mixed with sharp pain. Notably, the subcortical and cortical limbic regions were deactivated while the somatosensory cortices and the dorsal raphe nucleus in the brainstem were activated during acupuncture *deqi*. When *deqi* was mixed with sharp pain ($N = 4$), the result was predominantly activation, but fewer regions were affected. Sensory control ($N = 5$) resulted in activation of fewer regions. Abbreviations: BA—Brodmann area; SCC—subcallosal cortex; VMPF—ventromedial prefrontal cortex; and SII—secondary somatosensory cortex.

row, and Fig. 7). The lateral hemispheres demonstrated extensive signal attenuation that was most marked in lobules V, VI and VIIA (Table 2C; Fig. 5, bottom row, Fig. 7). The fastigial nucleus and the interpositus nucleus on the right and the dentate nucleus on both sides showed moderate signal attenuation (Table 2D).

The rest of the brain likewise demonstrated widespread signal decreases (Table 1; Figs. 3 and 4). The most robust signal changes were seen in the amygdala, hippocampus, parahippocampus, subgenual and pregenual cingulate and the ventromedial prefrontal cortex. Other limbic, cortico-limbic, subcortical and brainstem structures showed similar responses, including the septal area, nucleus accumbens, hypothalamus, posterior cingulate, anterior insula, anterior caudate, temporal pole, frontal pole, ventral tegmental area, substantia nigra, reticular formation and pontine nuclei (Tables 1A, B and D; Figs. 3 and 4). The dorsal raphe nucleus showed signal increase instead (Table 1D). In the thalamus, the posterior division showed signal attenuation; other divisions demonstrated significant individual variations in their response patterns (Table 1B). SII and the topographic representation of the lower extremity in the paracentral lobule showed signal increases. The premotor area (BA 6) and the inferior parietal lobe (BA 40), however, showed signal decreases (Table 1C).

Acupuncture: deqi mixed with pain (N = 4)

Signal intensities showed changes in both directions with a predominance of signal increases in the cerebro-cerebellar and limbic systems. Although the sharp pain was only mild to moderate and much shorter in duration than the *deqi* sensations, the entire cerebro-cerebellar and limbic systems showed a predominance of activation. However, the extent of hemodynamic response was more limited than in the *deqi* group (Tables 1 and 2; Figs. 3–5).

In the cerebellum, lobule V of the anterior vermis was activated, consistent with recent reports of activation by pain as evidenced by fMRI (Dimitrova et al., 2003; Helmchen et al., 2003). No changes were detected in the posterior vermis, which is functionally more limbic. Most the regions related to cognition in both the medial hemispheres – lobules VI, VIIA and VIIB – and in the lateral hemispheres – lobules V, VI, VIIA Crus I, VIIIA and VIIIB – showed activation as opposed to deactivation in *deqi* (Table 2, Fig. 5). No significant changes were detected in the nuclear structures of the cerebellum and brainstem.

The cerebral circuit likewise showed a mixed response with predominance of signal increases. (Table 1; Figs. 3 and 4). Effects were conspicuously absent in the major structures of the limbic system, including the amygdala, hippocampus, septal area and hypothalamus. Paralimbic structures as the parahippocampus and the cingulate cortex showed signal increases, contrasting with signal decreases in *deqi*. The temporal pole, frontal pole, prefrontal cortex and inferior parietal lobe showed signal changes in both directions. The premotor cortex showed signal increase in mixed sensations, contrasting with the signal decrease seen in the *deqi* subjects. Activation of the secondary somatosensory cortex (SII)

Table 2
FMRI signal changes in the cerebellum – *Deqi* vs. *Deqi*+pain

		Acupuncture										Sensory					Functions
		Deqi					*Deqi* + **pain**										
		N = 11					N = 4					N = 5					
		Talairach			Δ	P	Talairach			Δ	P	Talairach			Δ	P	
		x	y	z	signal	value	x	y	z	signal	value	x	y	z	signal	value	
A. Vermis		(mm)			(%)	(log)	(mm)			(%)	(log)	(mm)			(%)	(log)	
III	R	0	-38	-13	-0.28	-4.23											
	L	0	-38	-13	-0.28	-4.23											
IV	R																
	L																
V	R																
	L	-2	-56	-2	- 0.21	-3.67	-1	-62	-11	0.38	-3.93						
VI	R	2	-71	-14	-0.20	-3.34						2	-71	-24	0.43	-4.90	
	L	-2	-71	-15	-0.31	-5.61						-2	-71	-24	0.43	-4.90	
VII A	R											2	-68	-24	0.29	-4.60	
	L											-2	-68	-24	0.43	-7.14	
VII B	R	4	-71	-35	-0.40	-5.36											
	L																
VIII A	R	2	-62	-34	-0.36	-6.65											
	L																
VIII B	R	9	-62	-45	-0.47	-4.10											
	L																
IX	R	1	-53	-37	-0.26	-3.71											
	L	-3	-47	-30	-0.24	-4.03											
X	R	0	-44	-30	-0.43	-5.60											
	L	0	-44	-30	-0.43	-5.60											
B. Medial hemispheres																	
III	R																
	L																
IV	R	26	-32	-23	-0.35	-4.05											SI
	L	-8	-38	-10	-0.33	-3.92											
V	R	15	-50	-13	-0.29	-5.74						10	-59	-9	0.37	-3.45	
	L	-16	-50	-12	-0.17	-3.49						-9	-59	-12	0.25	-3.95	
VI	R											8	-74	-15	0.46	-6.50	
	L						-15	-64	23	0.62	-5.62	-10	-74	-21	0.38	-5.46	
VIIA Crus I	R	15	-83	-25	-0.47	-5.94	12	-83	-21	0.68	-8.65	5	-79	-24	0.60	-5.85	
	L	-15	-86	-20	-1.04	-5.14	-12	-86	-24	0.53	-3.56	-7	-80	-22	0.67	-5.85	
VIIA Crus II	R	18	-77	-33	-0.38	-5.70						8	-83	-22	0.57	-3.88	
	L	-12	-71	-31	-0.27	-3.68	-12	-79	-24	0.36	-4.80	-4	-83	-25	0.91	-3.97	
VII B	R	13	-77	-43	-0.69	-6.70	6	-71	-37	0.68	-5.04	2	-76	-37	-0.75	-4.75	
	L	-10	-68	-30	-0.32	-3.98						-7	-74	-37	0.42	-3.97	
VIII A	R																
	L											-16	-65	-37	0.43	-8.29	SII
VIII B	R	10	-59	-36	-0.41	-6.16						9	-59	-37	0.26	-3.39	
	L	-14	-50	-54	-1.23	-9.60											
IX	R	6	-65	-40	-0.41	-6.42	11	-53	-55	1.06	-4.38						Balance
	L	-6	-53	-51	-0.50	-6.82						-9	-47	-37	0.31	-6.06	
X	R																
	L	-22	-38	-38	-0.63	-8.57											
C: Lateral hemispheres																	
V	R	37	-38	-27	-0.58	-8.30	33	-32	-27	0.90	-5.70	28	-38	-24	0.46	-5.70	
	L	-28	-32	-24	-0.74	-8.04											
VI	R	35	-38	-28	-0.54	-8.90	43	-45	-22	0.97	-10.18	34	-63	-20	0.79	-8.83	
	L	-21	-43	-29	-0.57	-7.49						-24	-64	-18	0.35	-8.70	
VIIA Crus I	R	37	-44	-30	-0.30	-4.29	34	-74	-19	1.45	-3.66	38	-65	-22	1.32	-7.47	
	L	-36	-38	-34	-0.77	-3.58	-41	-71	-27	0.55	-4.48	-44	-71	-22	1.49	-4.56	
VIIA Crus II	R											27	-70	-37	0.30	-3.40	
	L	-31	-66	-43	-0.49	-4.05						-30	-80	-34	0.42	-3.60	

Table 2 (*continued*)

VIIB	R											30	-71	-43	0.48	-5.87
	L											-22	-71	-43	0.77	-7.46
VIIIA	R	20	-62	-49	-0.44	-5.18	38	-44	-40	-1.10	-4.42	24	-56	-40	0.25	-3.45
	L	-36	-44	-43	-0.47	-5.88	-32	-44	-43	0.58	-3.66	-25	-62	-42	0.33	-5.38
VIIIB	R	28	-35	-37	-0.17	-4.85										
	L	-35	-47	-43	-0.23	-4.02	-29	-38	-46	1.28	- 3.42	-18	-54	-40	0.27	-4.23
D: Deep nuclei																
Fastigial	R	2	-50	-24	-0.23	-3.96										
	L															
Interpositus	R	10	-56	-28	-0.23	-3.27										
	L															
Dentate	R	10	-56	-28	-0.28	-4.03										
	L	-19	-47	-27	-0.23	-4.27						-14	-62	-30	0.38	-4.17

Sensorimotor, posture, balance

Autonomic control, emotions

Cognition

Quantified fMRI signal changes in the cerebellum, with comparisons, data sources and color coding the same as described for Table 1. The functions of different regions of the cerebellum, as described in the literature, are listed on the right (Makris et al., 2003). The regions related to emotion and autonomic control in the vermis and to cognition in the medial and lateral hemispheres showed significant changes in signal intensity. The predominant response of the cerebellum correlated with those of the cerebrum and brainstem, in that signal attenuation was predominant with *deqi* and enhancement with sharp pain and sensory control (see Table 1).

was stronger than in the *deqi* group, but the signal change remained under 1%, less than values reported in pain literature (Table 1C). In concert with the stronger somatosensory cortex activation in mixed sensations than in *deqi*, signal increase was observed in a large subset of the cerebro-cerebellar and limbic structures in mixed sensations instead of the signal decrease seen in *deqi* (Hui et al., 2003).

Sensory control (N = 5)

Superficial tactile stimulation over the acupuncture point ST 36 also produced predominant signal enhancement in the cerebro-cerebellar circuit. In the cerebellum signal increases were observed in lobules VI and VIIA of the vermis (sensorimotor), lobules V–IX of the medial hemispheres (sensory, cognition, balance) and lobules V–VIIIB of the lateral hemispheres (cognition). The response was mostly bilateral, being most prominent in VIIA Crus I of the lateral hemispheres, with signal increase reaching 1.4%. The dentate nucleus showed a slight signal increase on the left (Table 2; Figs. 3–6). Interestingly, the ventromedial prefrontal cortex demonstrated predominant signal attenuation. Changes were conspicuously absent in other limbic regions such as the amygdala, hippocampus, septal area and hypothalamus (Table 1; Figs. 3–5).

Discussion

This is the first systematic study of the effects of traditional Chinese acupuncture on the entire human cerebro-cerebellar circuit that correlates the patterns of hemodynamic response and psychophysical response with each other. The central effects of acupuncture needle stimulation demonstrated in this study differ markedly from those of conventional peripheral nerve stimulation conducted on the tibial nerve of the leg or on the median nerve of the hand that innervate the acupuncture points ST 36 and LI 4, respectively. Studies using EEG, MEG and fMRI reported that non-painful electrical or other sensory stimulation of the tibial nerve in normal subjects activated the somatosensory, motor, premotor, posterior parietal and cingulate cortices, the thalamus and the cerebellum (Smith et al., 2003). The same pattern of response has been described more extensively for the median nerve at the wrist (Desmedt and Bourguet, 1985; Korvenoja et al., 1999). The activation of the cingulate and cerebellum by tactile stimulation (Smith et al., 2003) is in agreement with our results on tactile control stimulation at the acupoint. We are unaware of reports of deactivation by non-acupuncture oriented stimulation.

Anatomical and electrophysiological studies of acupuncture point structure and function show that both manual and electro-acupuncture stimulate the same peripheral nerves as conventional electrical stimulation. However, in marked contrast to the signal enhancement in these non-acupuncture oriented studies, manual acupuncture at ST 36 in the present study and at LI 4 in an earlier study (Hui et al., 2000) elicited widespread and synchronized signal decreases in the cerebro-cerebellar circuit, especially marked in the limbic system. Except for conditions that require stronger stimulation to achieve excitatory action, acupuncture at the main points generally produces anti-stress and anti-anxiety effects (Mann, 1992). These findings suggest that modulation of the cerebro-cerebellar and limbic system activity may constitute an important pathway of acupuncture action. Acupuncture action involves the interplay between multiple neurotransmitters and modulators. Correlation of the distribution and the known functions of these mediators in the cerebro-cerebellar and limbic systems with the hemodynamic response to acupuncture suggests that the down-regulation of dopaminergic and norepinephrinergic tone coupled with the up-regulation of the serotonergic tone during the procedure may initiate a cascade of reactions that results in the more delayed effects of acupuncture. Validation would require investigation with methodologies that can probe into the fundamental processes underlying the BOLD response.

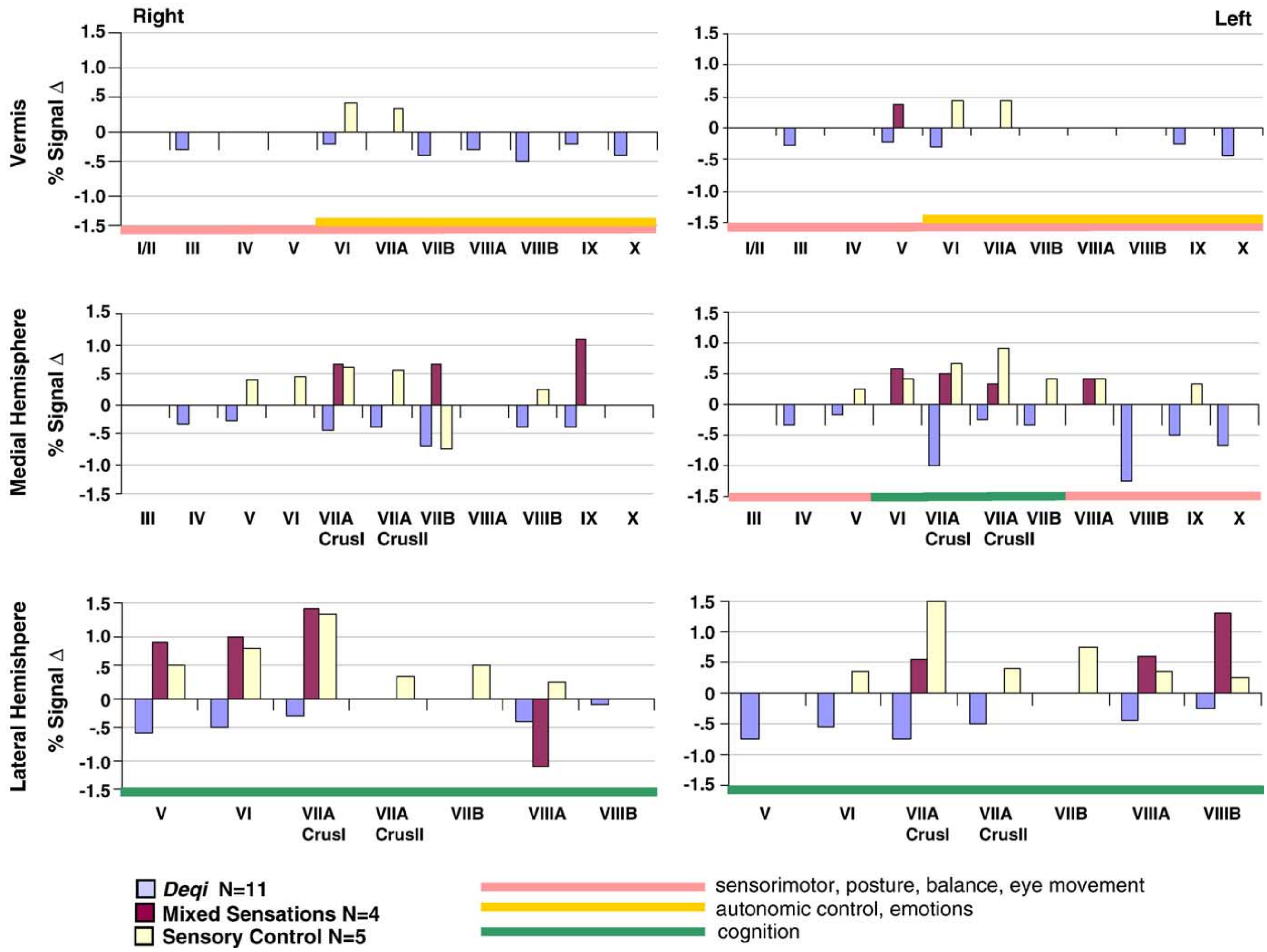

Fig. 5. Comparison of hemodynamic response of the cerebellum between different categories of psychophysical response. The magnitude of signal change in the cerebellum for different stimulations at acupoint ST 36 as listed in Table 2. The known functionality of the different regions of the cerebellum are labeled with a color code at the bottom of the chart. Acupuncture with *deqi* resulted in widespread signal decreases in the vermis and hemispheres. When *deqi* was mixed with pain, response was more limited. A large subset of regions related to pain, cognition, affect and autonomic control were activated. Sensory control demonstrated predominant signal increases, with minor signal decrease in the posterior medial hemisphere.

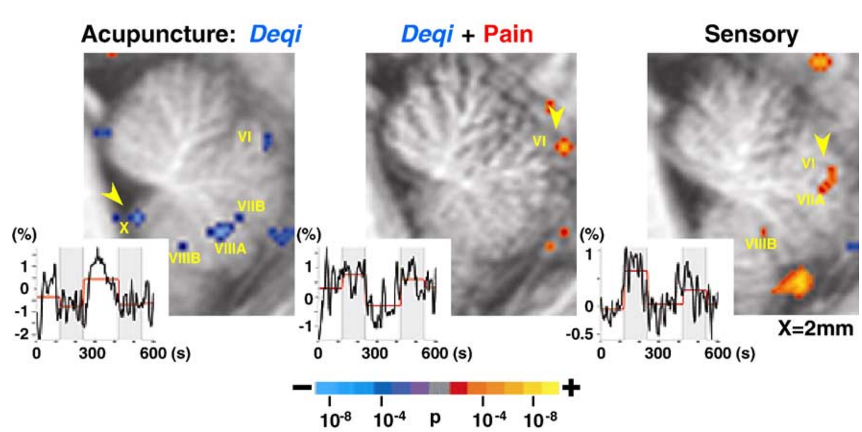

Fig. 6. Close-up of group average cerebellum from Fig. 3A, as described therein. The slices are taken sagittally at 2 mm to the right of the midline in Talairach space. The time course shown in the lower left corner of each image is taken from the location denoted by the arrowhead in that image. The vertical axis of the time course denotes percent signal change, and the horizontal axis the time within the experimental run, in seconds. Zero percent signal change is placed at the average activity level for the three epochs with the needle at rest. The shaded columns represent the periods of needle manipulation. (Left) Acupuncture with *deqi* sensations (N = 11) resulted in widespread signal decreases, including lobules VI, VIIB, VIIIA and B, IX and X. (Center) Acupuncture with *deqi* plus sharp pain (N = 4) resulted in signal increase in lobules V, VI. (Right) Sensory control (N = 5) demonstrated signal increase in lobules VI, VIIA and VIIIB.

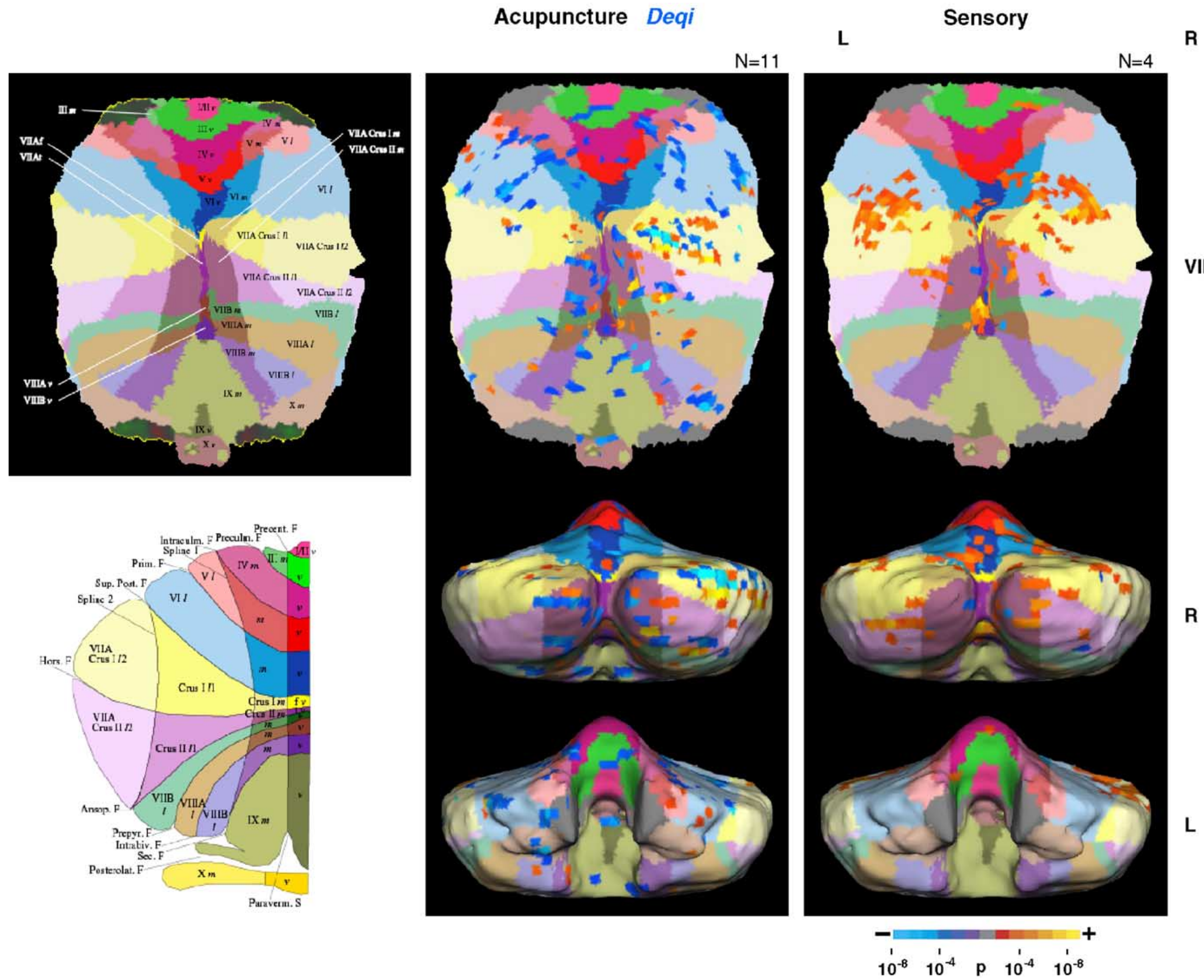

Fig. 7. fMRI signal changes on the exterior surface are overlaid onto the cerebellum overlaid onto its reconstructed surface, both 3D and flattened. (Top row) Flattened surface. (Middle Row) 3D caudal view. (Bottom row) 3D rostral view. The parcellation units are color coded according to the figure in the left column, adapted from Makris et al. (2003). Gray regions denote the cerebellar peduncles. Acupuncture with *deqi* (middle column) produced prominent signal changes with a predominance of negative BOLD response. The signal decreases in the vermis can be seen in both the caudal and rostral views of the 3D cerebellum. Sensory control (right column) showed regions with signal increase that were concentrated in lobules VI and VII.

Cerebro-cerebellar and limbic systems

The cerebro-cerebellar system consists of a feedforward limb and a feedbackward limb that form the cerebro-ponto-cerebellar-thalamo-cortical loop (Fig. 8) (Schmahmann, 2000b, 2001). The cerebro-pontine pathway of the feedforward limb carries outputs from the primary and association cortices (sensorimotor, prefrontal, posterior parietal, superior temporal, dorsal extrastriate) and the limbic/paralimbic structures (posterior parahippocampus, cingulate gyrus, mammillary nucleus) to the brainstem (periaqueductal gray, pontine nucleus, reticular formation, dorsal raphe, locus caeruleus). The ponto-cerebellar tract then connects the secondary neurons of the pontine structures with the vermis, hemispheric zones and deep nuclei of the cerebellum. In the feedback limb, the cerebello-thalamic pathway connects the dentate and interpositus nuclei of the cerebellum with the thalamus via the red nucleus, and the thalamo-cortical projection connects the secondary neurons in the thalamus with the motor and non-motor cerebral cortices (Dum and Strick, 2003; Gonzalo-Ruiz and Leichnetz, 1990; Schmahmann, 2000a).

In addition to the cerebro-ponto-cerebellar-thalamo-cortical loop, the cerebellar cortex and deep nuclei are connected to the hypothalamus and mamillary bodies via direct connections (Dietrichs, 1984; Haines and Dietrichs, 1984). Physiological and behavioral studies demonstrated intimate linkage between the cerebellar vermis and fastigial nucleus, the so-called ‘limbic cerebellum,’ with major cerebral limbic structures, the septal nuclei, amygdala and hippocampus. The violent rage induced in the cat and rat by electrical stimulation of the ‘limbic cerebellum’ could be abolished by severing their connections with the amygdala and hypothalamus (Heath et al., 1978; Rasheed et al., 1970; Snider and Maiti, 1976). The fastigial nucleus of the cerebellum is also connected with he ventral tegmental area, locus caeruleus and dorsal raphe nucleus in the brainstem that send diffuse dopaminergic, norepinephrinergic and serotonergic projections to the limbic system, basal ganglia and cerebral cortex

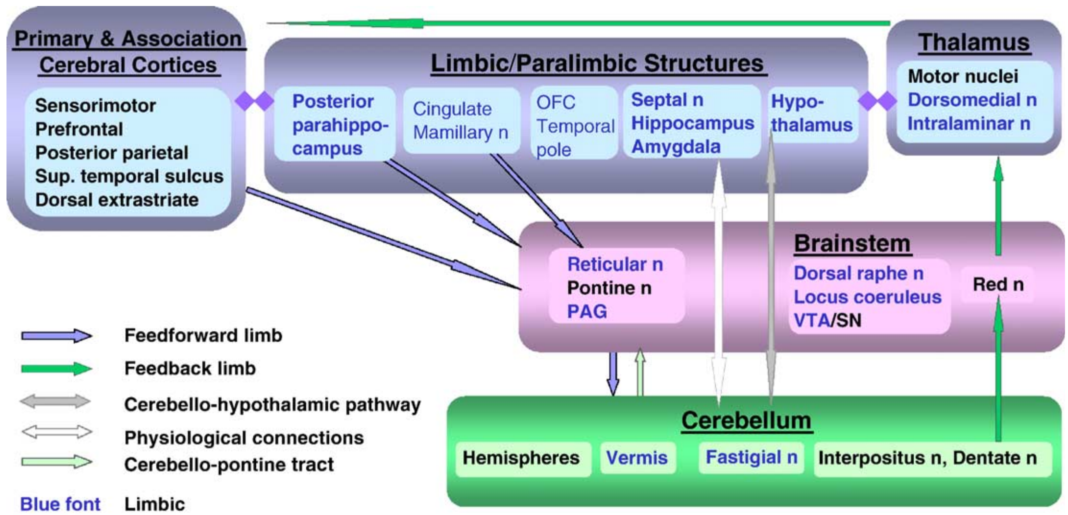

Fig. 8. The cerebro-cerebellar system, or the cerebro-ponto-cerebellar-thalamo-cortical circuit, as described by Schmahmann (2000a, 2001). The feedforward limb starts with the cerebro-pontine pathway, which carries outputs from the cerebral sensorimotor and association cortices and from the limbic and paralimbic structures to the pontine structures of the brainstem. Secondary neurons connect the pontine structures with the vermis, hemispheres and deep nuclei of the cerebellum via the pontocerebellar tract. The feedback limb consists of projections from the dentate and interpositus nuclei of the cerebellum to the thalamus via the red nucleus in the brainstem, and of the thalamocortical tract from the thalamus to motor and non-motor cerebral cortices. The cerebellum also projects back to the brainstem via the cerebello-pontine pathway (Schmahmann, 2000a). It is connected directly with the hypothalamus through the cerebello-hypothalamic pathway (Haines and Dietrichs, 1984). Close physiological linkages exist between the vermis, the nodular lobule and the fastigial nucleus of the 'limbic cerebellum with the amygdala, hippocampus, and septal nucleus of the cerebral limbic system' (Heath and Harper, 1974). Abbreviations: n—nucleus; sup—superior; PAG—periaqueductal grey; VTA—ventral tegmental area; SN—substantia nigra.

(Dempesy et al., 1983; Ikai et al., 1992; Marcinkiewicz et al., 1989; Nieoullon et al., 1978; Snider et al., 1976).

The limbic system plays a central role in the regulation and integration of sensorimotor, autonomic, endocrine and immunological functions, as well as cognition and affect. It is responsible for major functions of the organism, such as the maintenance of homeostasis, feeding and drinking activities, defense and attack behaviors and sexual reproduction. In the telencephalon, it comprises the septal and pre-optic regions, the amygdala, hippocampus, parahippocampus, anterior thalamus, entorhinal cortex, cingulate gyrus and the bed nucleus of the stria terminalis. The diencephalons contain the pineal gland, habenula, hypothalamus and zona incerta. The periaqueductal grey, reticular formation, raphe nuclei and a number of midline structures in the brainstem as well as the vermis and fastigial nucleus in the cerebellum also belong to the limbic system. These structures are interconnected by short and long fiber bundles, forming a structural and functional continuum, the so-called "limbic system–midbrain circuit" (Nauta, 1972; Nauta and Haymaker, 1969). Given these widespread anatomical, neuropharmacologic and functional connections in the limbic continuum, their concerted response to acupuncture stimulation should not be surprising.

BOLD fMRI signal changes

Acupuncture with deqi. During acupuncture at ST 36 with *deqi* sensations, signal decreases predominated in the cerebro-cerebellar and limbic systems. Signal increases were uncommon, primarily limited to the somatosensory cortex and the serotoninergic dorsal raphe nucleus. Other investigators have also reported signal decreases in the amygdala, hippocampus, caudate, anterior cingulate and putamen (Wu et al., 1999), and in the posterior cingulate, superior temporal gyrus, putamen/insula (Kong et al., 2002) during manual acupuncture at LI 4 or ST 36. A recent study on transcutaneous electric acupuncture point nerve stimulation at ST 36 and SP 6 (Spleen 6, above the medial malleolus) demonstrated signal decreases in the amygdala and hippocampus that were related to the analgesic effect of acupuncture in the same subjects (Zhang et al., 2003). Results on the hypothalamus and nucleus accumbens, however, show discrepancy in the literature. Whereas we observed signal decreases in these regions with acupuncture *deqi* at both LI 4 (Hui et al., 2000) and at ST 36 in this study, others have reported signal increases, using fMRI for acupuncture at ST 36 (Wu et al., 1999), and using PET for acupuncture at LI 4 (Hsieh et al., 2001).

These discrepancies could be attributed to the different needling techniques and stimulation intensities employed by the different investigators and whether sharp pain, even of moderate degree and short duration, occurred during the stimulation. The bidirectional rotation with even motion technique was adopted in this study, while the needle rotation was coupled with thrusting and lifting in Wu's study (Wu et al., 1999). The subjective sensations as well as the clinical effects of acupuncture are known to be influenced by needling techniques (Cheng, 1997). As regards the PET studies (Hsieh et al., 2001), the spatial resolution did not permit definitive delineation of the hypothalamic activation from the large area of activation that extended to the midbrain and brainstem. A recent report on the central effects of manual acupuncture at PC 6 (pericardium 6) proximal to the wrist demonstrated only positive activations (Yoo et al., 2004). We concur with the authors that a major difference between the nature and the clinical indications of the acupoints being studied could be a likely cause. PC 6 is primarily used for nausea, vertigo and cardiac arrhythmias, while ST 36 and LI 4 are commonly used for analgesia, stress and disorders of multiple systems. They comprise the most frequently used acupoints in traditional Chinese acupuncture (Napadow et al., 2005). Moreover, PC 6 is located in tendinous structures just above the wrist while LI 4 and ST 36 are located in muscles. PC 6 is more superficial and more prone to cause overt pain. The distribution of receptors and afferent neural fibers may differ with tissue type.

The down-regulation of limbic system activity by acupuncture was demonstrated by electro-physiological studies in non-human primates as early as 3 decades ago. Using chronically implanted microelectrodes in the squirrel monkey, both manual and electro-acupuncture at LI 4 and ST 36 decreased the cell firing rate and increased the interspike interval in the septal area, amygdala and anterior cingulate, while thalamic activity in the parafascicular and ventral posteromedial nuclei remained unaffected. It was proposed that the limbic response rather than the thalamic response could be the neurophysiologic correlate of acupuncture analgesia (Jacobs et al., 1977). However, most acupuncture research to date has been devoted to acupuncture analgesia, using rats as animal models and focusing on sensory perception and opioid peptides rather than on the affective dimension of pain and the limbic system. Perhaps the modulatory effects of acupuncture as evidenced by modern in vivo neuroimaging in humans may serve to revive this important old concept.

The magnitude of signal change observed in acupuncture *deqi* was small, generally less than 1%, compared with the 2–4% activation by visual stimulation or other sensory tasks reported in the literature (Kwong et al., 1992). The smaller response suggests that acupuncture, unlike noxious insults and pharmacological agents, may act within physiological limits. This could explain in part why acupuncture treatment generally causes fewer side effects than medications, particularly potent analgesics.

Acupuncture with mixed sensations (deqi and pain). When acupuncture *deqi* is mixed with sharp pain, the hemodynamic response associated with the sensations may oppose or neutralize each other. The net outcome may depend on which sensation prevails. Structures that show signal attenuation in acupuncture *deqi* such as the amygdala, hippocampus, cingulate cortex, temporal pole, frontal pole are known to show signal enhancement by pain and noxious insults (Becerra et al., 1999; Casey et al., 1994; Coghill et al., 1994; Craig et al., 1996; Davis, 2000; Jones et al., 1991; Price, 2000; Talbot et al., 1991). In this study, the stronger activation of the somatosensory cortices in the mixed sensations than in the *deqi* group can be attributed to the presence of pain. The limbic cortices, including the temporal pole, frontal pole, ventromedial prefrontal cortex, however, showed mixed responses. Importantly hemodynamic response was conspicuously absent in a large subset of the limbic structures, including the amygdala, hippocampus, the subgenual, pregenual and posterior cingulate cortex, the septal area and the anterior insula. The two opposing effects, deactivation elicited by *deqi* and activation elicited by pain, might have neutralized each other. An alternative explanation could be the failure of acupuncture to deactivate these limbic structures and suppress the pain. The predominant effect of pain was manifest in the activation of the parahippocampus, middle cingulate cortex (Table 1) and cerebellum (Table 2). Recent fMRI studies on pain have demonstrated extensive activation of the anterior vermis (Dimitrova et al., 2003; Helmchen et al., 2003). Consistent with these reports, activation was observed in lobule V of the anterior vermis in mixed sensations, in contrast to deactivation of multiple anterior vermal lobules in *deqi*. Signal increases were also widespread in the hemispheric regions related to cognition. Overall, the results suggest that in mixed sensations, the central effects of pain prevailed, exhibiting an integrated response with predominance of activation over deactivation in the cerebro-cerebellar and limbic systems (Table 2, Fig. 5). The contrast in the direction of signal change between different types of psychophysical response was even more striking when sharp pain occurred in the absence of *deqi*. In earlier studies when the sensitivity to needling was not pre-tested prior to scanning, one subject experienced pain without *deqi* during acupuncture at ST36. Multiple lobules of the anterior vermis and hemispheric zones exhibited robust activation with signal reaching 1–5% in the cerebro-cerebellar and limbic systems (Hui et al., 2003).

Control stimulation. Tactile stimulation over the acupoint using the same paradigm elicited a predominance of signal increase in the entire brain, in marked contrast to signal decrease observed in acupuncture *deqi* (Tables 1 and 2). The deactivation of the paralimbic ventromedial prefrontal cortex and adjoining subgenual cingulate is interesting (Table 1A). It is reported that attention and anticipatory anxiety could induce deactivation of the ventro-medial prefrontal cortex (Gusnard et al., 2001; Simpson et al., 2001a,b). The subjects were instructed to pay attention to the sensations that they might experience during the stimulation so that they could describe them during the interview at the end of the scan. This could constitute an attention task and contribute to the deactivation of these regions. Another possible explanation could be the down-regulation of the brain activity from its default state by the soothing rhythmic tactile stimulation (Raichle et al., 2001). The activation of the dentate nucleus of the cerebellum by tactile stimulation was in agreement with reports that the deep cerebellar nuclei were activated by sensory stimulation in the absence of any motor task (Gao et al., 1996). Activation of the hemispheric zones of the cerebellum that participate in cognition and emotional processing was also in agreement with an increasing number of clinical and neuroimaging reports that relate the cerebellum to thought and emotional control in health and in disease (Ritvo et al., 1986; Schmahmann and Sherman, 1998; Townsend et al., 2001; Wallesch and Horn, 1990; Weis et al., 2004).

Negative BOLD response. The signal intensity in BOLD fMRI is the net result of the neuronal activity that determines oxygen consumption and the hemodynamic response to the changes in O_2 demand. Most fMRI studies are based on the detection of a positive BOLD response. Here, we have demonstrated a robust negative BOLD response to acupuncture that correlates with *deqi* sensations. The redistribution of blood to activated regions with a greater demand, known as "blood stealing" or "physiological steal", has been proposed for negative activations that are coupled to positive responses in the proximity (Shmuel et al., 2001, 2002). However, the absence of positive response in adjacent regions in acupuncture rules out such an explanation for the negative response in this task. Although hemodynamic changes independent of the local changes in neuronal activity cannot be completely ruled out, we favor reduction of neuronal activity due to a task-specific deactivation of these brain regions. Specifically the task is acupuncture stimulation that induces *deqi* sensation. This proposal is based on an increasing number of reports that cognitive processes, emotion, attention and goal-directed behaviors can induce functional deactivations as measured by BOLD fMRI, PET and electrophysiological methods (Ferris et al., 2004; Friston et al., 1991; Frith et al., 1991; Ghatan et al., 1998; Goldapple et al., 2004; Hutchinson et al., 1999; Liotti et al., 2000; Lustig et al., 2003; Raichle et al., 2001; Shulman et al., 1997; Simpson et al., 2001a,b). Various causes have been proposed, including regional inhibition (Frith et al., 1991), task-specific inhibition (Ghatan et al., 1998), task-independent suppression of tonic activity (Shulman et al., 1997), an overactive default state (Lustig et al., 2003; Raichle et al., 2001) or intrinsic to the activation state (Hutchinson et al., 1999). Regions in the medial aspect of the brain, including the ventromedial prefrontal cortex, cingulate cortex (subgenual and posterior divisions), the medial parietal lobe and the amygdala, are prone to negative activation by tasks that involve attention and cognition (Gusnard et al., 2001; Lustig et al., 2003; Raichle et al., 2001; Simpson et al., 2001a,b). These regions converge with the ones that demonstrate negative activation in acupuncture.

Evidence is emerging that establishes the negative BOLD response as a marker of neuronal deactivation rather than a local phenomenon attributed to "blood stealing". A visual stimulus that stimulated primary visual cortex in one hemisphere caused extensive suppression in the other hemisphere (Shmuel et al., 2003a,b; Smith et al., 2004). The BOLD response, cerebral blood flow (CBF) and cerebral metabolic rate of oxygen consumption ($CMRO_2$) signals increased in the contralateral and decreased in the ipsilateral cortex in response to a hand motor task. The relative changes in the CBF and $CMRO_2$ were linearly related. The findings characterized the hemodynamic and metabolic down-regulation accompanying neuronal inhibition and indicated neuronal deactivation as the cause of the negative BOLD response (Stefanovic et al., 2004). The negative BOLD response during human sleep correlated with EEG changes that suggest true cortical deactivation (Czisch et al., 2004). It has also been shown by neuroimaging that sedation attenuates cerebral blood oxygenation in contrast to augmentation by stimulants (Kleinschmidt et al., 1999). Among several possible interpretations, neuronal deactivation is the most plausible neurocorrelate of a sustained negative BOLD response. Whether the deactivation results from down-regulation of excitatory or up-regulation of inhibitory forces remains to be explored. This would require other experimental approaches to probe into the neurochemical processes that underlie the hemodynamic response.

Neurotransmitters/neuromodulators. Of the multiple mediators that may participate in acupuncture action, the endogenous opioid peptides are best known, but the latency that is required for a peptidergic neurotransmitter to respond to a stimulus cannot explain the rapid rise and fall of fMRI signals during the 10-min scan. Among different possible interpretations, we propose that the diffuse neuromodulators widely distributed in the cerebro-cerebellar and limbic systems, especially dopamine, may play an important role. Dopamine is the neuromodulator of highest concentration in the limbic system (Cooper et al., 2002), and its transporters occur in abundance in the posterior–inferior lobules of the cerebellar vermis (Melchitzky and Lewis, 2000). Animal data acquired by different experimental approaches indicated that acupuncture suppressed the synthesis and/or release of dopamine induced by pain (Shi et al., 1986), cocaine (Yang et al., 2001) or alcohol (Yoon et al., 2004). A recent fMRI study demonstrated that electroacupuncture at the forepaw of rats reversed the activation of the nucleus accumbens, striatum, cingulate and amygdala induced by amphetamine. The normalization of the hemodynamic response was accompanied by a return of the elevated dopamine content to the baseline levels as evidenced by microdialysate assays (Chen et al., 2001, and private communications). Convergence of these animal data with the prominent deactivation of the richly dopaminergic regions in the limbic network in human brains suggests that regulation of the dopaminergic tone could be an important mechanism of acupuncture action. Serotonin, another monoaminergic neuromodulator, is reported to play an important role in acupuncture analgesia (Han, 1998). It participates in the control of Purkinje cell glutamate release in the cerebellum (Darrow et al., 1990; Kitzman and Bishop, 1997) and its deficiency is implicated in cerebellar ataxia (Trouillas et al., 1997). The signal increase of the median raphe nuclear groups observed in the present and an earlier study (Napadow et al., 2005) is consistent with serotonergic activation. The deactivation of the subgenual cingulate and the reticular formation observed by fMRI is also consistent with reports of sympathetic tone down-regulation by acupuncture (Cao et al., 1983; Haker and Bjerring, 2000). Thus, the patterns of hemodynamic response are in accord with the current knowledge of acupuncture effects on the monoaminergic mediators and autonomic system function, a finding that requires validation with studies of larger sample size.

Acupuncture and the limbic aspects of the cerebro-cerebellar system. The negative BOLD response to acupuncture with *deqi* is among the most robust deactivations reported for a task-specific stimulus. In addition to signal decrease in the multiple limbic/paralimbic structures and limbic cortices in the cerebrum (such as the amygdala, hippocampus, parahippocampus, cingulate, septal area, temporal pole, frontal pole and ventromedial prefrontal cortex), the same direction of signal change was observed in the ventral tegmental area and reticular formation of the brainstem, and in the vermis, deep nuclear groups and hemispheric zones of the cerebellum. The vermis and fastigial nucleus are known as the "limbic cerebellum" and the hemispheric regions are involved in cognition and emotional control.

The modulatory effects of acupuncture with *deqi* may bear resemblance to that of cognitive behavioral therapy (CBT) in bipolar disorder patients. In patients who responded to CBT, resting-state ^{18}F-deoxyglucose PET demonstrated signal attenuation in the cortico-limbic regions, while in patients who received conventional medication treatment, signal enhancement was observed instead (Goldapple et al., 2004). The authors proposed that CBT might mobilize the neurophysiological system to self-regulate and restore the homeostasis of cognitive and affective functions. Acupuncture could likewise mobilize the neurophysiological system to self-regulate and restore the balance of multisystem functions.

Psychophysical sensations. Sensations of numbness, pressure, aching and fullness are conducted by type II and III ($A\beta$, $A\gamma$, $A\delta$) myelinated nerve fibers with a faster conducting rate, while temperature, soreness and pain are conducted by slower conducting type III $A\delta$ or type IV (C) fibers (Gardner et al., 2000; Lu et al., 1979; Wang et al., 1985). The high prevalence and rating of pressure, aching, numbness and tingling in the acupuncture *deqi* group are consistent with the major involvement of the myelinated and fast-conducting type II and III afferents in muscle tissues. As the stimulation increases in intensity, more type III $A\delta$ and some type IV or C fibers become involved, giving rise to sensations of warmth, coolness, soreness and pain. The dull pain induced by noxious insults and carried by fine C fibers is generally preceded by sharp pain (Basbaun and Jessell, 2000). The pain-related structures are activated as demonstrated by PET or fMRI (Casey et al., 1994; Coghill et al., 1994; Davis et al., 1997; Jones et al., 1991). In marked contrast, the dull pain in acupuncture *deqi* often occurs in the absence of sharp pain or precedes the sharp pain. It is typically associated with deactivation of the limbic and pain-related structures. This interesting dichotomy raises the question whether the fiber bundles carrying dull pain in acupuncture differ from those carrying classical second pain. Acupuncture may predominantly stimulate type II and type III fibers to elicit the typical psychophysical and hemodynamic responses that are distinct from responses to noxious insults. There is a spectrum of type IV or C fibers with gradations in size and impulse transmission rate that transmit different sensations in addition to pain perception (Craig, 2004). It is possible that the dull pain in *deqi* may involve some C fibers which are not as small and not as slow conducting as the C fibers that carry second pain. Involvement of the very fine fibers in noxious stimulations would activate the so-called diffuse noxious inhibitory system and elicit a positive BOLD response.

Limitations

Sample size. The data sets used in this report are derived from an ongoing investigation that is of much larger sample size. Thus, the preliminary results from this study will require validation with additional data. However, acupuncture-imaging studies of similar or smaller size have yielded interesting and innovative findings and provided impetus to further investigations. We are the first to examine in detail the effects of acupuncture on the entire brain, and correlate the visualized central effects with the psychophysical response; the novel findings warrant further investigation. As part of a large study, acupuncture was administered to ST36, LI4 and LV3 in a randomized order in the same subject. Possible interference among the points cannot be established or ruled out with this small sample size.

Confounding factors when deqi is mixed with pain. To compare the central effects of acupuncture *deqi* and noxious stimulation, it would be desirable to have a group of subjects who respond with overt pain in the absence of *deqi*. However, the study was not designed to use matched groups for comparison. The specific aim of the investigation was to study the effect of typical acupuncture with *deqi.* Sharp pain was caused only unintentionally. The subjects were grouped *in retrospect* according to the sensations that they experienced during the procedures. None of the subjects experienced pain without *deqi* and only a small number had *deqi* mixed with pain. In the mixed sensations group, the effects of *deqi* and sharp pain confound each other, but we can still glean the differences between them. The overwhelmingly negative activation with *deqi* is in stark contrast to the predominant activation with mixed sensations. One could design an experiment that blocks the conductance of type II and type III fibers related to *deqi* (Pomeranz, 2001) and administer forceful needle manipulation that would stimulate type IV or C fibers to produce overt pain. This would require a separate study. In this retrospective study, we can only draw on the abundant evidence in the literature on the positive activation of the cerebro-cerebellar and limbic systems by noxious insults as a basis for comparison.

Experimental control. The design of an optimal control for acupuncture stimulation remains unresolved (Ernst and White, 1997). It is known that acupuncture performed to sites that are not located on meridians can have varying degrees of physiological and clinical effects. Therefore, we chose to deliver superficial tactile stimulation to the skin surface as a non-invasive control at the acupoint rather than invasive needling at a non-acupoint. Lying supine in the magnet bore, the subjects could not see the devices being used to conduct the tests on their extremity. They were not aware which test was being performed, the sensory control or the real acupuncture. When control stimulation is given in an environment where the subject's view of the site of stimulation is not blocked, a retractable 'placebo' acupuncture needle would be more appropriate (Streitberger and Kleinhenz, 1998).

Temporal effects and acupuncture techniques. The neural circuits and neurotransmitter systems involved in acupuncture action may be time dependent. How the early events demonstrated in this study translate into the cumulative effects and health benefits of acupuncture require further investigation. The findings reported here apply to the commonly used manual acupuncture technique of needle rotation that is 'balanced in reinforcing and reducing action' in terms of traditional Chinese medicine (Cheng, 1997). Studies on other techniques of needle manipulation are in progress.

Artifactual activation. Although modern neuroimaging has opened up avenues for the non-invasive monitoring of the human brain, susceptibility artifacts and motion artifacts limit the interpretation of the hemodynamic response. The structures at the base of the brain that border the nasal cavity are particularly subject to susceptibility artifacts. Cerebrospinal fluid movement due to cardiac pulsations affect the brainstem. In addition, residual intersubject registration errors of the functional data can cause confounds of signal detection, particularly in the brainstem and cerebellum. Although our findings are in large part consistent across the studies conducted to-date, special experimental designs and methods are being devised to address these problems in our investigation.

Conclusions

This is the first detailed study of the cerebellum in conjunction with the rest of the brain that maps the extensive effects of acupuncture on the cerebro-cerebellar and limbic systems. Results indicate that acupuncture elicits an integrated response from multiple levels of the brain that is dependent on the psychophysical response. Negative activation of the cerebrocerebellar and limbic systems is typically seen with *deqi.* The hemodynamic response correlates with the current knowledge of acupuncture effects on the monoaminergic systems. We propose that these neuromodulators, in particular dopamine, may play an important role in acupuncture action, a hypothesis that warrants investigation.

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