Neural Pathway Interference by Retained Acupuncture: A Functional MRI Study of a Dog Model of Parkinson's Disease

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Keywords

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SUMMARY

Objective: The aims of this study were to investigate the interference of the brain activation during a passive movement task (PMT) by retained acupuncture at the ST 36 acupoint and to compare these effects between normal brain and Parkinson's disease (PD) brain.

Methods: Functional magnetic resonance imaging (fMRI) techniques have been used to study neurophysiology in animals. Eight healthy beagle dogs were divided into two groups of four dogs each, a normal control group and a PD model group. PD was induced by intravenous injection of 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-HCl. During fMRI, the PMT was performed in the right tarsal joint during three different sessions, which consisted of PMT only, PMT while an acupuncture needle was inserted at the ST 36 acupoint, and PMT while needle was inserted at a sham point. **Results and discussion:** Standard veterinary neurological examination was performed on dogs with MPTP-induced PD. A homogeneous grade similar to human PD patients was evident in all dogs. The fMRI study showed that insertion of the acupuncture needle at acupoint ST 36 significantly affected the proprioceptive brain activation by decreasing blood oxygenation level-dependent signal intensity in basal ganglia, limbic system, and cerebellum. Compared with normal and PD brain, we suggest that acupuncture at ST 36 has different modulation effects depending on the pathologic condition of the brain. The study provides evidence of the potential clinical applications of retained acupuncture at ST 36 for rehabilitation therapy of PD patients.

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Introduction

Acupuncture is widely used for the treatment of many neuronal disorders in oriental medicine by inserting needles into specific body points called acupoints [1,2]. Recent developments in neuroimaging techniques have enabled the demonstration by researchers in the fields of oriental medicine and neuroscience of the specific correlation of acupuncture with the central nervous system [3]. The blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) technique, which estimates brain activity indirectly by measuring alterations of blood flow, blood volume, and oxygen [4,5] to scan brains of subjects undergoing needling, has been used to show the correspondence of different acupoints with different cerebral areas and conditioned reaction [3].

Most of the previous acupuncture-related fMRI studies have investigated neurodegenerative disorders, such as Parkinson's disease (PD), and have focused on acupoint specificity [6,7] or functional architecture of brain connectivity networks in acupuncture [8], to provide the pathophysiological basis for the efficacy of acu-

puncture. Several studies have found evidence of acupuncture-mediated improvement of the pathology of motor disorders, especially in PD, by the restoration of homeostasis in the nigrostriatal dopaminergic pathway [3,6,9].

Despite these reports of acupuncture-related benefits in motor disorders, no studies have yet investigated the effects of retained acupuncture on the brain when external stimuli are applied, even though the external stimulations, such as passive movement or pain, are usually applied to motor disorder patients as diagnostic methods [10–15].

In this study, we hypothesized that the activated brain region with proprioceptive sensory stimuli can be affected by the retention of acupuncture needle at a certain acupoint. Furthermore, if acupuncture indeed improves symptoms of motor disorder by restoring homeostasis in the dopaminergic pathway, we also hypothesized that the functional changes of proprioceptive stimuli in the pathologic brain also can be affected.

To investigate these hypotheses, the present study had two purposes. The first was to investigate the interference of the brain activation during a passive movement task (PMT) by acupuncture

needle insertion at a certain acupoint. The second was to compare these effects between normal and pathologic brains. For these purposes, we focused on PD because it is most intensively studied neurodegenerative disease that shows motor disorder as a major symptom [10,11]. The Zusanli (ST 36) was chosen because it is one of most intensively studied acupoints for treating PD [6,16,17]. As an appropriate disease model animal, we chose the 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxic-induced PD canine model. The brain size of dogs is easily comparable with human studies, in contrast to small experimental animals like mice and rats that are usually used as PD animal models.

Materials and Methods

Animal Preparation

Eight beagle dogs (15.8 ± 4.5 months, weighing 11.1 ± 1.2 kg) without sex discrimination (six males and two females) were divided into two groups ($n = 4$ in each group): the normal control (NC) group and the PD model (PDM) group. To minimize the sex differential effects, a female dog was included in both groups. Before inducing PD in the PDM group, the dogs were determined to be normal by physical and hemodiagnostic (complete blood

count) examinations and blood chemistry. The dogs were fasted for 12 h prior to the MRI experiment but were permitted free access to water. Each dog was individually housed according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and a protocol (KU-08076) approved by the Institutional Laboratory Animal Care and Use Committee of our local institute.

MPTP-induced PD Model

MPTP-HCl (Sigma-Aldrich, St Louis, MO, USA) was used as a neurotoxic agent for inducing PD in the dogs [18]. The animal modeling procedure followed a previous description [19]. MPTP-HCl was diluted with 0.9% saline (0.05 mg/mL solution) to prevent acute systemic damage or death [18]. The dogs in the PDM group were injected three times with 2.5 mg/kg MPTP-HCl every second day by a syringe pump (1.25 mg/kg per h). By the last injection, the animals had difficulty standing and exhibited clinical features of PD, including resting tremor, rigidity, freezing episodes, decreased spontaneous movements, and barking. Neurological examinations of the PDM group were performed prior to and every day of the dosing period. Standard veterinary neurological examinations to assess changes in the level of consciousness, gait,

Figure 1 Photographs of the anatomical location of the acupoint and sham acupoint (A), and diagrams of experimental paradigms (B). (A) Guide wires were connected on the top of the acupuncture needle to distinguish between acupoints. (B) The needle was inserted with Deqi 1 min before applying passive moving task (PMT). The PMT was performed for 30 seconds after a 30-second rest period. MO, PMT motion only; TA, PMT with acupuncture of true acupoint; SA, PMT with acupuncture of sham point.

posture, and brain stem reflexes were graded on a scale of 0–3 (absent to normal) [20,21].

fMRI Stimulation Paradigm

During fMRI, PMT was performed in the right tarsal joint during three different sessions, which consisted of PMT only (MO), PMT while an acupuncture needle was inserted in the right ST 36 acupoint (TA), and PMT while the needle was inserted in a sham point (SA). To perform PMT, the animals were placed in a sternal position and the hind limbs were extended backward. The experimenter held the tarsal joint of the animal's right hind limb to generate proprioceptive stimulation. A sound signal every 30 second alerted the experimenter to switch from the baseline state (without movement) to the activation state (with movement), and this procedure was repeated four times. The paradigms of this study are shown in Figure 1B. Each session ran 4 min with 80 image volumes. During the TA session, a stainless steel acupuncture needle was inserted approximately 1 cm deep into the Zusanli acupoint (ST 36), which is near the knee joint of the hind limb lateral to the anterior tubercle of the tibia (Figure 1A). The needle was manually rotated clockwise and counterclockwise to generate Deqi, which is generally accepted as the characteristic acupuncture effect [8,9]. PMT was then performed. During the SA session, the same needle was inserted 1.5 cm lateral of ST 36 in the same manner as the true acupuncture insertion. This sham acupuncture point was used as a control. PMT was performed after the sham insertion. To prevent an influence on the pain signal by the insertion of the needle at the true or sham acupoint, the MO session was always performed first in all animals. After that, TA and SA sessions were alternatively performed one by one.

fMRI Acquisitions

MR imaging was performed on an Achieva 3 Tesla MRI system (Philips Healthcare, Best, The Netherlands) with an eight-channel sensitivity encoding (SENSE) knee coil, which was equipped with echo-planar imaging (EPI) hardware. During the imaging procedures, the dogs were sedated by intramuscular injections of medetomidine (40 mg/kg; Pfizer Korea, Seoul, Korea) and anesthetized with intravenous injections of ketamine (15 mg/kg; Pfizer Korea). The fMRI signals were collected using a T2*-weighted, gradient-echo EPI sequence. The detailed acquisition parameters were as follows: repetition time (TR) = 3 second, echo time (TE) = 35 ms, flip angle = 90°, field of view (FOV) = 120 mm × 114 mm, matrix size = 68 × 60 reconstructed to 128 × 128 pixels, number of dynamic scans = 80, voxel size = 1.78 mm × 1.84 mm reconstructed to 0.94 mm × 0.94 mm, SENSE factor = 1.8, number of slices = 25, slice thickness = 4 mm without a gap between slices, slice orientation = transverse, slice scan order = interleaved, number of dummy scans = 2 and EPI factor = 37. In addition, anatomic images were acquired with a T2-weighted turbo spin echo (TSE) sequence of the dog brain in the axial, coronal, and sagittal directions with the following parameters: TR = 4540 ms, TE = 80 ms, matrix size = 156 × 144, voxel size = 0.71 × 0.84 × 2.00 mm³ reconstructed to 0.42 × 0.42 × 2 mm³, FOV = 110 mm × 121 mm, TSE factor = 16, flip angle = 90° and refocusing angle = 120°. Scan time for each direction was 1.5 min.

Creation of the Study-Specific Template

To analyze the acquired fMRI data, a study-specific dog brain template was created in the following way. A good representative and symmetric T2-weighted structural image was selected and aligned in a standard stereotaxic space along the anterior commissure and posterior commissure [22]. An initial template was created whereby the images were co-registered, and a single mean image was produced. All T2-weighted structural images were then spatially normalized to this mean image with a voxel size of 1 × 1 × 1 mm, and a second mean image was made from these normalized images. The second mean image was then smoothed

Figure 2 Regional activation resulting from the passive moving task of each session in the normal control (A) and the Parkinson disease model (B) group. Red areas represent the activated brain regions (rest < PMT). In contrast, blue areas represent the deactivated brain regions (rest > PMT). Main affected brain regions were (1) basal ganglia, (2) pons, (3) cerebellum, (4) limbic system, (5) temporal lobe, and (6) thalamus. MO, passive moving task (PMT) motion only; TA, PMT with acupuncture of the true acupoint; SA, PMT with acupuncture of the sham point.

using an isotropic Gaussian kernel with a full-width at half-maximum (FWHM) of 2 mm, which resulted in a well-defined study-specific template.

The second mean image from the procedure of the study-specific template was skull-stripped using the Brain Extraction Tool of the MRIcro software (<http://www.mccauslandcenter.sc.edu/mricro>) and segmented with the Oxford Centre for Functional MRI of the Brain (FMRIB)'s Automated Segmentation Tool (FAST) from the FMRIB Software Library (FSL: <http://www.fmrib.ox.ac.uk/fsl>) into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF). All scans were segmented into GM, WM, and CSF with the Statistical Parametric Mapping software (SPM8: <http://www.fil.ion.ucl.ac.uk/spm>). Finally, the mean segmented images were smoothed using an isotropic Gaussian kernel with a FWHM of 2 mm.

fMRI Data Analysis

SPM8 software was used for the preprocessing and analyzing of fMRI data. The first three EPI images of each dog were discarded to reduce T1 equilibrium effects. The other EPI images were realigned to the first volume, and the mean functional EPI image was created. The mean functional image was then co-registered to a structural image. To make inter-individual comparisons and combine data from several subjects, all images were spatially normalized onto the previously created study-specific template with a voxel size of 1 mm × 1 mm × 1 mm. Finally, the functional images were smoothed with a Gaussian kernel (FWHM = 4 mm × 4 mm × 6 mm) to increase the sensitivity and statistical validity.

Table 1 Comparison of passive moving tasks (PMT) in the normal control (NC) group

Region	Side	MO				TA				SA			
		Base < PMT		Base > PMT		Base < PMT		Base > PMT		Base < PMT		Base > PMT	
		KE	Z score	KE	Z score	KE	Z score	KE	Z score	KE	Z score	KE	Z score
<i>Cortex</i>													
Rostral sylvian gyrus	R							58	2.85				
Middle suprasylvian gyrus	L							4	2.78				
Middle suprasylvian gyrus	L							254	3.19				
Rostral sylvian gyrus	R											18	2.85
												5	2.61
Caudal sylvian gyrus	L							20	2.74			301	3.31
Rostral suprasylvian gyrus	R									26	2.86		
Rostral ectosylvian gyrus	L			8	2.84								
	R									8	2.8		
Middle ectosylvian gyrus	R			15	3.14							6	2.85
				9	2.78								
Caudal ectosylvian gyrus	R	18	3.28										
Piriform lobe	L			98	4.06								
	R			15	3.16								
Ectomarginal gyrus	L							15	2.87				
								10	2.84				
	R							7	2.83				
<i>Limbic and Sublobar</i>													
Olfactory peduncle	L							161	3.27				
Caudate nucleus	L			58	3.23							38	3.26
	R							36	3.16				
								22	2.91				
Hippocampus	L											69	2.9
	R									44	3.05		
Cingulate gyrus	L			5	2.72							230	3.25
	R							124	2.93				
Thalamus	R							24	2.92			7	2.72
<i>Brain stem</i>													
Reticular formation	R	5	2.84										
<i>Cerebellum</i>													
Hemisphere	R	20	2.96										
Vermis								32	2.93	11	2.72		
Peduncle	R					6	2.93						
	R							27	3.81				

P value < 0.005 without multiple comparisons; extent threshold = 4 voxels; K_E = size of clusters. MO: PMT only, TA: PMT while acupuncture needle is inserted at the ST 36 acupoint, and SA: PMT while acupuncture needle is inserted at a sham point.

Statistical Analyses

In the individual analyses, cerebral activations during PMT relative to baseline conditions were calculated by 1st-level specification in SPM8 with our stimulation paradigm. The skull-stripped brain image was used for the explicit mask to clear the activation on the extra-brain structures. We obtained contrast maps for both activated and deactivated areas of each experimental condition. Significant differences were accepted at a threshold of $P < 0.005$ without multiple comparison correction, and the extent threshold was four voxels. For the group comparison, the data were analyzed using the factorial design specification in SPM8. The statistical analysis of each condition was compared with each other. The

statistical parametric maps (SPMs) with two factors and three conditions were generated using full factorial design. We focused our study on the group analysis and report here only the results of the group study.

To investigate the BOLD signal changes during PMT, activated or deactivated contrasts of each sessions in the NC group and the PDM group were generated to calculate on a sample *t*-test using the full factorial design. Therefore, the group effect of MO, TA, and SA of NC group and PDM group was obtained. For the within-group analysis, positive and negative *t*-contrasts of two sessions, which were coupled by MO and TA, MO and SA, and TA and SA, were generated to calculate repeated-measure ANOVA using the full factorial design. To find the relative differences

Table 2 Comparison of passive moving tasks (PMT) in the Parkinson disease model (PDM) group

		MO				TA				SA			
		Base < PMT		Base > PMT		Base < PMT		Base > PMT		Base < PMT		Base > PMT	
Region	Side	KE	Z score	KE	Z score	KE	Z score	KE	Z score	KE	Z score	KE	Z score
Cortex													
Postcruciate gyrus	R	7	2.81										
Marginal gyrus	L			318	3.61								
				37	3.26								
Rostral suprasylvian gyrus	L			13	2.97								
	R			34	3.1								
Rostral sylvian gyrus	L			238	4.3								
Middle suprasylvian gyrus	L	25	3.57										
Middle suprasylvian gyrus	R							5	2.72				
Middle sylvian gyrus	R			484	3.74								
Rostral ectosylvian gyrus	L											18	2.89
	R											80	3.04
Caudal ectosylvian gyrus	L							5	2.71				
	R			150	3.42								
Caudal sylvian gyrus	L			49	3.13								
	L			7	2.7								
Splenial gyrus	R			258	3.6								
Occipital gyrus	R			24	2.83								
Endomarginal gyrus	R			102	3.79								
Piriform lobe	L			13	3.14								
				11	2.86								
Limbic and Sublobar													
Olfactory peduncle	R			7	2.8							11	2.73
Caudate nucleus	L							413	3.52			63	3.04
	R			2636	4.93								
Cingulate gyrus	L			8	2.71							42	2.77
Amygdala body	R			77	3.42								
Brain stem													
Reticular formation	L											6	2.95
	R							11	3.05			4	2.81
Caudal colliculus	L			294	3.73								
	R			51	3.31								
Crus cerebri	R			10	2.99								
Cerebellum													
Peduncle	L			9	2.96			14	3.02				
	R			114	3.87			24	3.51				

P value < 0.005 without multiple comparisons; extent threshold = 4 voxels; K_E = size of clusters. MO: PMT only, TA: PMT while acupuncture needle is inserted at the ST 36 acupoint, and SA: PMT while acupuncture needle is inserted at a sham point.

between the NC group and the PDM group, we performed two-sample *t*-test using full factorial design for between-group comparisons for the three conditions of MO, TA, and SA separately. Significant differences of all statistical results were accepted at a threshold of $P < 0.005$ without multiple comparison correction, and the extent threshold was four voxels. The locations of activated areas were shown by superimposition on axial sections of three-dimensional rendered images of a well-scanned subject, which was normalized with the brain templates.

Results

Neurological Examinations

Neurological examinations of the PDM group showed that MPTP induced a depressed state of consciousness and a rapid decline of motor performance within 3 days of treatment. Although the score of the brain stem reflexes of the first dog was decreased from 3 to 2 after the first and second MPTP treatment, by the end of the treatment period, the score for consciousness was decreased from 3 to 2, and the score for gaiting and placing was decreased from 3 to 1 in all dogs.

One Sample *t*-Test of the fMRI Results

NC Group

For the MO session, comparisons of the baseline states and activation states showed that PMT increased the BOLD signal intensity in the reticular formation of the pons and cerebellum, but decreased the BOLD signal intensity in the bilateral temporal lobes, piriform lobe, contralateral basal ganglia, and limbic system. For the TA session, PMT increased the BOLD signal intensity in the right cerebellar peduncle, but decreased the BOLD signal intensity in the olfactory peduncle, limbic system, basal ganglia, thalamus, parietal-temporal-occipital association cortex, and cerebellum. For the SA session, PMT increased the BOLD signal intensity in the rostral area of the ipsilateral temporal lobe, hippocampus, and cerebellum, but decreased the BOLD signal intensity in the bilateral temporal lobes, contralateral basal ganglia, limbic system, and ipsilateral thalamus. The detailed areas are shown in Figure 2A and are listed in Table 1.

PDM Group

For the MO session, comparisons of the baseline states and activation states showed that PMT slightly increased the BOLD signal intensity in the primary somatosensory area, but decreased the BOLD signal intensity in the limbic system, basal nuclei, thalamus, caudal colliculus, parietal-temporal-occipital association cortex, and cerebellum. For the TA session, PMT did not increase the BOLD signal intensity in any region, but decreased the BOLD signal intensity in the ipsilateral parietal lobe, contralateral temporal lobe, basal ganglia, pons, and cerebellum. For the SA session, PMT did not increase the BOLD signal intensity in any region, but decreased the BOLD signal intensity in the bilateral temporal lobes, basal ganglia, limbic system, and pons. The detailed areas are shown in Figure 2B and are listed in Table 2.

Within-group Analysis of the fMRI Results

In the NC group, comparison of brain activity between MO and TA determined that the MO session showed different activation in the left rostral suprasylvian gyrus, left middle ectomarginal gyrus, right ectomarginal sulcus, left olfactory peduncle, and cerebellum. In contrast, the TA session showed different activation in left piriform lobe. Comparison between MO and SA revealed that the MO session showed different activation in the left caudal ectosylvian gyrus and left hippocampus. In contrast, the SA session showed

Figure 3 Results of within-group analysis for the comparisons of differential activated brain area between each session in normal control (A) and the Parkinson disease model (B) group. The two sessions separated by a slash at the left side of each brain image indicate comparisons of activated differences between the left and right side. Red area represents brain regions that were more active during session at left compared to session at right (left > right). In contrast, blue areas represent brain regions that were more active during sessions at right compared to session at left (left < right). Main activated brain regions were (1) piriform lobe, (2) cerebellum, (3) hippocampus, (4) caudate nucleus, (5) thalamus, (6) medulla of occipital lobe, and (7) pons.

different activation in the right middle suprasylvian gyrus, left medullar of parietal lobe, bilateral piriform lobe, and cerebellum. Comparison between TA and SA indicated that the TA session did not show any different activation, but that the SA session showed different activation in the bilateral medullar of the parietal lobe, right middle ectomarginal gyrus, fornix, and cerebellar peduncles.

In the PDM group, comparison of brain activity between MO and TA determined that the MO session showed different activation in the left middle ectomarginal gyrus. In contrast, the TA session showed different activation in the left presylvian sulcus, left medullar of ectosylvian sulcus, right ectosylvian sulcus, right marginal gyrus, left middle ectomarginal gyrus, bilateral cingulate

Table 3 Comparison of differential activated brain area between each session in normal control (NC) and the Parkinson disease model (PDM) groups by within-group analysis

Group	Region	Side	MO > TA		MO < TA		MO > SA		MO < SA		TA > SA		TA < SA	
			K _E	Z score	K _E	Z score	K _E	Z score	K _E	Z score	K _E	Z score	K _E	Z score
NC	<i>Cortex</i>													
	Middle suprasylvian gyrus	R							11	2.87				
	Rostral suprasylvian gyrus	L	39	3.08										
	Medullar of Parietal lobe	L							5	2.89			63	3.18
		R											80	3.08
	Caudal ectosylvian gyrus	L					8	2.88						
	Piriform lobe	L			32	3.45			70	4.08				
		R							14	3.09				
	Middle ectomarginal gyrus	L	5	2.75										
		R											9	2.63
	Ectomarginal sulcus	R	14	3.11										
	<i>Limbic and Sublobar</i>													
	Olfactory peduncle	L	46	3.29										
	Fornix	L											18	3.18
	Hippocampus	L					20	3.01						
	<i>Cerebellum</i>													
	Hemisphere	R	27	3.04										
	Vermis								8	2.81				
	Peduncle	L	8	3.74									39	3.00
		R							7	3.08			9	3.15
PMD	<i>Cortex</i>													
	Presylvian sulcus	L			14	2.86								
	Medullar of Parietal lobe	R							5	2.72				
	Medullar of ectosylvian sulcus	L			9	2.92								
	Ectosylvian sulcus	R			5	2.94								
	Piriform lobe	R							21	2.89				
	Marginal gyrus	R			23	2.99								
	Middle ectomarginal gyrus	L	4	2.73	211	3.64								
	(connected with cingulate gyrus)					3.51								
	Caudate ectomarginal gyrus	R							21	2.84				
	Coronal sulcus	L							7	2.76				
	Cingulate gyrus	R			17	3.05								
	<i>Limbic and Sublobar</i>													
	Olfactory bulb	L							5	2.71				
	Olfactory peduncle	R			64	3.19								
	Thalamus	R			190	3.45								
	Caudate nucleus	R							33	2.89				
	Lateral geniculate nucleus	L			18	2.98								
	Pons	R							11	2.88				
	<i>Cerebellum</i>													
	Hemisphere	R			5	2.63								
	Vermis				4	2.73								

P value < 0.005 without multiple comparisons; extent threshold = 4 voxels; K_E = size of clusters. MO: PMT only, TA: PMT while acupuncture needle is inserted at the ST 36 acupoint, and SA: PMT while acupuncture needle is inserted at a sham point.

gyrus, right olfactory peduncle, right thalamus, left lateral geniculate nucleus, and cerebellum. Comparison between MO and SA revealed that the MO session did not show any different activation, but that the SA session showed different activation in the right medullar of the parietal lobe, right piriform lobe, right caudate ectomarginal gyrus, left coronal sulcus, left olfactory bulb, right caudate nucleus, and right pons. Comparison between TA and SA did not show any different activation. The detailed areas of the within-group analysis are shown in Figure 3 and listed in Table 3.

Two-Sample *t*-Test of the fMRI Results (Between-Group Analysis)

For the MO session, compared to the NC group, the PDM group showed higher BOLD signal intensities in the ipsilateral motor-sensory area, but lower BOLD signal intensities in the contralateral somatosensory area, basal nuclei, limbic system, thalamus, bilateral parietal-temporal-occipital association cortex, and cerebellar peduncle. For the TA session, the PDM group exhibited higher BOLD signal intensities in the contralateral motor-sensory area, bilateral parietal lobes, thalamus, limbic system, and cerebellar vermis, but lower BOLD signal intensities in the cerebellar peduncle and pons. For the SA session, the PDM group did not show higher BOLD signal intensities in any region, but showed lower BOLD signal intensities in the contralateral motor-sensory area, ipsilateral somatosensory area, limbic system, and hypophysis. These areas are shown in Figure 4 and listed in Table 4.

Discussion

The present study demonstrates the influence of retained acupuncture needles at ST 36 in the proprioceptive neural pathway of normal dogs and PD model dogs. The contralateral primary sensory cortex, primary motor cortex, and supplementary motor area at the cortical regions [22,23] and ipsilateral cerebellum and contralateral posterior putamen at the subcortical regions [22] are the most common activated areas when passive movements are applied. In this study, however, these regions were not activated during PMT application in the normal dogs. The probable causes of these results will be discussed in the limitations portion of this article. Here, we will discuss the activated or deactivated area in subcortical regions, which include the dopaminergic pathway, to determine the differences between each session and group.

Interruption of the Neural Pathway of Needle Insertion at the ST 36 Acupoint

The first major purpose of the present study was to determine the change of brain activation interfered in the proprioceptive neural pathway by retention of the acupuncture needle at ST 36. The results from the NC group showed that neural activity of the cerebellum and dopaminergic pathway including the basal ganglia and limbic system were decreased during the TA session and were partially increased during the SA session (Figure 2A), while the PMT stimulation alone activated the reticular formation and cerebellum. In addition, the results of within-group analysis showed MO session has larger activated area than TA session, while it has

Figure 4 Results of the comparisons of the regional activation during a passive moving task (PMT) between the normal control (NC) and Parkinson disease model (PDM) groups for the PMT motion only (A, MO), PMT with acupuncture of the true acupoint (B, TA), and PMT with acupuncture of the sham point (C, SA). Red areas represent the activated brain regions in PDM group compared with NC group (PDM > NC); in contrast, blue areas represent the deactivated brain regions in PDM group compared with NC group (PDM < NC). Main affected brain regions were (1) basal ganglia, (2) thalamus, and (3) cerebellum; MO, passive moving task (PMT) motion only; TA, PMT with acupuncture of the true acupoint; SA, PMT with acupuncture of the sham point.

smaller activated area than SA session (Figure 3A). Especially, the TA session showed no different activation in the limbic system and cerebellum, while both MO and SA sessions showed activation in these areas. Hui *et al.* [24] demonstrated that manual acupuncture stimulation at ST 36 can elicit widespread and synchronized BOLD signal decreases in the cerebro-cerebellar and limbic systems, similar to the present results, and suggested that the modulation of the cerebellar pathway and limbic system activity may constitute an important pathway of acupuncture action. As the ST 36 acupoint is commonly used for acupuncture analgesia, the authors also suggested that the limbic response could be the neurophysiological correlate of acupuncture analgesia.

Different from Hui *et al.* [24], the present study stimulated acupoint by Deqi only once, and the inserted needle was retained as PMT was performed. Therefore, our results demonstrate that retained acupuncture also can modulate the limbic system activity during PMT. We suggest that retained acupuncture at ST 36 interrupts the neural pathway when proprioceptive stimuli are applied by performing PMT.

Neural Pathway Interference by Needle Insertion Loses Acupoint Specificity in the PDM Group

The present results from the PDM group showed that most of the dopaminergic pathway areas were decreased as PMT was performed. Compared to the NC group, during PMT, the number of

Table 4 Comparison of passive moving task (PMT) activities between the normal control (NC) and the Parkinson disease model (PDM) groups

		MO				TA				SA			
		NC > PDM		NC < PDM		NC > PDM		NC < PDM		NC > PDM		NC < PDM	
Regions	Side	KE	Z score	KE	Z score	KE	Z score	KE	Z score	KE	Z score	KE	Z score
Cortex													
Prorean gyrus	L							9	2.8				
Postcruciate gyrus	R			12	2.86								
Rostral suprasylvian gyrus	L											5	2.74
	R											31	2.87
Rostral sylvian gyrus	R	12	2.96										
Marginal gyrus	R							4	2.71				
Middle ectosylvian gyrus	R	88	3.82										
Middle suprasylvian gyrus	L							21	3.1				
								9	2.78				
Splenial gyrus	L	41	3.19										
	R	212	3.34										
Occipital gyrus	R	5	2.7										
Ectomarginal gyrus	L	38	3.13					10	2.79				
Endomarginal gyrus	R	59	3.06										
Limbic and Sublobar													
Olfactory peduncle	L							18	3.15				
								6	2.74				
	R					28	2.91						
Caudate nucleus	R	846	4.4										
	L		3.31										
			3.27										
		33	3.05										
Putamen	R	17	3.04										
Cingulate gyrus	R									7	2.81		
Central tegmental tract	L	6	2.87										
	L	82	3.25										
Thalamus	L		2.65										
	R							5	2.92				
Brain stem													
Reticular formation	L					6	2.7			7	2.86		
	R	32	3.16										
		10	2.95										
Hypophysis										16	2.84		
Red nucleus	R	25	2.98										
Spinal trigeminal nucleus	R	50	3.46										

P value < 0.005 without multiple comparisons; extent threshold = 4 voxels; K_E = size of clusters. MO: PMT only, TA: PMT while an acupuncture needle is inserted at the ST 36 acupoint, and SA: PMT while an acupuncture needle is inserted at a sham point.

deactivated areas in the PDM group was larger than the number of activated areas, indicative of damage to the dopaminergic neurons. In addition, unlike the NC group, the needle insertion at the ST 36 acupoint and sham point produced less of a decrease of the BOLD signal in the dopaminergic pathway than the session in which only PMT was performed without needle insertion (Figure 2B). The results of within-group analysis also showed no different activation between the TA and SA sessions, while these sessions showed larger activation areas than the MO session (Figure 3B). These results support our suggestion that the interference of the proprioceptive neural pathway and analgesic effects by retained acupuncture abrogates the acupoint specificity in the PD brain.

Different Brain Modulation Effects of ST 36 Depend on Pathologic Conditions of the Brain

In the case of PMT alone (MO session), we observed relative deactivation in the basal nuclei, thalamus, and limbic system of PDM dogs compared to the NC group (Figure 4A). This result suggests that MPTP administration damaged the proprioceptive ability of the dog in the same way as that seen in PD [13,25,26]. On the other hand, in the TA session, we observed smaller signal differences than the signal differences of the MO session. Interestingly, despite the fact that most areas of the dopaminergic pathway were deactivated in the PDM group rather than the NC group, retention of the acupuncture needle at ST 36 enhanced the signal intensity

in these areas in PDM group (Figure 4B). Comparison of several acupuncture fMRI studies at ST 36 of normal brain [24,27] with those of PD brain [6,28] indicates that depending on the brain pathologic conditions, different brain modulations effects were produced, even if acupuncture needles had been inserted at the same acupoint. Thus, we suggest that our results provide evidence that acupuncture at ST 36 can have different modulation mechanisms depending on the pathologic conditions of the subject brain. In addition, in the case of the SA session, the BOLD signal intensity also displayed smaller differences than MO session, but all observed signal intensities were decreased in the PDM group (Figure 4C). We think these results are further evidence of the loss of acupoint specificity in the PD brain.

Limitations

This study had several limitations. These included the small number of subject and effects of anesthesia, which hindered definitive analysis of the cortical expression of proprioceptive stimuli. Especially, because BOLD fMRI is highly sensitive to subject movement, anesthesia is a necessary limitation imposed on research studies using experimental animals [29]. Several studies have indicated that fMRI can be carried out under anesthesia [29–32]. However, Ma and Leung [33] demonstrated that the effects of general anesthesia are potentiated by local inactivation of the limbic system, and Kim et al. [34] demonstrated that the anesthesia with ketamine and xylazine can change the thalamocortical connectivity of the brain. We suggest that our results indicate that the thalamocortical connectivity is changed by anesthesia. To clarify the effects of acupuncture compared with external stimulation on anesthetized animals, further fMRI studies to investigate the effects of anesthesia are needed. In addition, further studies should be performed with relatively large samples of animals.

Conclusions

In summary, our results show retained acupuncture needle at the ST 36 acupoint can interfere with proprioceptive brain activation by modulating the cerebellar pathway and limbic system. The interfering effect can cause the loss of acupoint specificity when the dopaminergic neurons in the brain are damaged. Acupuncture can have different modulatory effects that can influence the analgesic effects on normal brain and improve the kinesthesia in brain of PD, depending on the pathologic condition of the brain. Thus, our study provides evidence for potential clinical applications of retained acupuncture at ST 36 in rehabilitation therapy of PD patients. Furthermore, the MPTP-induced dog model of PD that was used in this study may be valuable in further functional studies of PD that use practical imaging instruments in human medicine for premedical research of human disease, such as long-term studies of several therapeutic effects including cell therapy, because the dog brain size is large and subcortical margins are clearer than small animals, such as rodents, that usually used in PD research.

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Conflict of Interest

The authors declare no conflict of interest.

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