Using MicroPET Imaging in Quantitative Verification of Acupuncture Effect in Ischemia Stroke Treatment

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Abstract

While acupuncture has survived several thousand years' evolution of medical practice, its function still remains as a myth from the view point of modern medicine. Our goal in this paper is to quantitatively understand the function of acupuncture in ischemia stroke treatment. We carried out a comparative study using the Sprague Dawley rat animal model. We induced the focal cerebral ischemia in the rats using the middle cerebral artery occlusion (MCAO) procedure. For each rat from the real acupuncture group ($n = 40$), sham acupoint treatment group ($n = 54$), and blank control group ($n = 16$), we acquired 3-D FDG-microPET images at baseline, after MCAO, and after treatment (i.e., real acupuncture, sham acupoint treatment, or resting according to the group assignment), respectively. After verifying that the injured area is in the right hemisphere of the cerebral cortex in the brain by using magnetic resonance imaging (MRI) and triphenyl tetrazolium cchloride (TTC)-staining, we directly compared the glucose metabolism in the right hemisphere of each rat. We carried out t-test and permutation test on the image data. Both tests demonstrated that acupuncture had a more positive effect than non-acupoint stimulus and blank control ($P < 0.025$) in increasing the glucose metabolic level in the stroke-injured area in the brain, while there

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was no statistically significant difference between non-acupoint stimulus and blank control ($P > 0.15$). The immediate positive effect of acupuncture over sham acupoint treatment and blank control is verified using our experiments. The long-term benefit of acupuncture needs to be further studied.

Keywords: Acupuncture Effect, Ischemia Stroke Treatment, MicroPET Imaging, Experimental Verification

Introduction

Acupuncture was developed based on the trial-and-error results of ancient Chinese medicine practitioners. It has survived the competition from varies of alternative techniques through the history and is one of the major components of traditional Chinese medicine. However, its mechanism largely remains illusive. The existence of the meridian channel (acupuncture is based on the meridian channel concept) is still under debate.

With the advances of modern imaging techniques, more and more studies have been carried out to validate the effect and to understand the mechanism of acupuncture in a quantitative manner. These imaging modalities include CT (Wang et al. (1993)), MRI (Li et al. (2004)), fMRI (Cho et al. (1998)), and PET (Huang et al. (2007)). The subjects in these studies include rats (Kim et al. (2009); Ren et al. (2008)), dogs (Syuu et al. (2003)), and human patients (Hopwood et al. (2008); Johansson et al. (2001)). Different set-ups including combinations of acupoints, time duration, types of acupuncture (e.g., manual acupuncture and electroacupuncture), and repeat patterns of acupuncture were used in these studies.

While many interesting findings have been reported in these studies, the mechanisms were not yet well understood and controversy results were often reported (Sze et al. (2002); Moffet (2006); Dhond et al. (2007)). One well-known example is that Cho et al. (Cho et al. (1998)) reported new findings of the correlation between acupoints and brain cortices using an fMRI-based approach in 1998. But they retracted their paper in 2006 for the reason of not being able to confirm the original findings in their follow-up studies. On the other hand, however, one promising finding by Witt et al. (Witt et al. (2005)) reported a positive effect of acupuncture in patients with osteoarthritis of the knee based on the Western Ontario and McMaster Universities Osteoarthritis (WOMAC) index from the questionnaires completed by about 300 patients. Certainly this finding needs to be further confirmed by more detailed studies.

In our opinion, indirect observations and potential involvements with irrelevant pathological conditions make the analysis and interpretation very difficult. To quantitatively understand the role of acupuncture, we need to have a statistically significant, biologically interpretable, and quantitatively repeatable measurement under the randomized trial setting. Here we decided to use the Sprague Dawley rat animal model to study the acupuncture effect in ischemia stroke treatment. We chose the FDG-microPET imaging technique (Phelps et al. (1979)) to measure the glucose metabolic level in the brain (Kornblum et al. (2000)), which is an important index of brain function (Schwartz et al. (1979); Chugani et al. (1987)). This study is based on the motivation of simplifying comparison and enhancing statistical significance and biological interpretability of findings. Concretely, our justifications are listed as follows:

- Rats has much a smaller diversity than human patients. Consequently, it is relatively easier to make homogeneous disease model using rats to avoid complications in pathology.

- Both fMRI and PET (including microPET) are popular modalities for functional imaging. Here we decided to use microPET in our experiments due to the following reasons:

  - It is difficult to follow the block design in fMRI for animal tests.
  - MicroPET directly provides a quantitative measurement related to metabolism.

  We are aware that microPET has a limited resolution. To overcome this drawback, we also use the magnetic resonance imaging (MRI) and triphenyl tetrazolium cchloride (TTC)-staining to help identify the injured brain area after ischemia stroke.

While we still follow the standard case-control comparison design, we try to simplify the comparison as much as possible. For example, many studies consider the accumulation timing effect of acupuncture and carry out the study for a period of several weeks or even up to a year. While this makes perfect sense clinically, factors such as pathological complications and changes in experimental conditions definitely will make the analysis more difficult. One obvious problem is that the sample size will not be enough to draw statistically significant conclusions for complicated experiments. Thus, we decided to only study the immediate effect of acupuncture.

Materials and Methods

Subjects

We used adult female Sprague-Dawley rats weighing 180 $\sim$ 280g. We randomly divided them into three groups:

1. Real acupuncture (RA) group. We used the combination of Baihui (GV 20) and Shuigou (GV 26) as target acupoints in this group (Hua et al. (1991); Li (2003)). These two acupoints were shown as red dots in Figure 1.

2. Sham acupoint (SA) treatment group. We used two non-acupoint locations (5 mm next to Baihui and Shuigou, shown as blue squares in Figure 1) as the target points for needling treatment [2].

3. Blank control (BC) group. We imaged the rats in this group without any acupuncture or sham acupoint treatment.

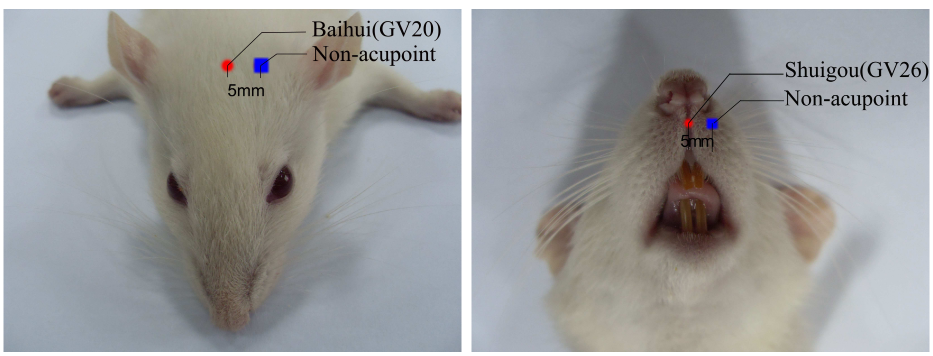

Figure 1: Locations of Baihui (GV20) acupoint (left image, the red dot on the head) and Shuigou (GV26) acupoint (right image, the red dot below the nose). Two non-acupoints with a distance of 5 mm to acupoints were chosen for sham acupoint treatment (marked as blue squares).

[2] The above locations are very close to two other acupoints. We will discuss more about this in the discussion section

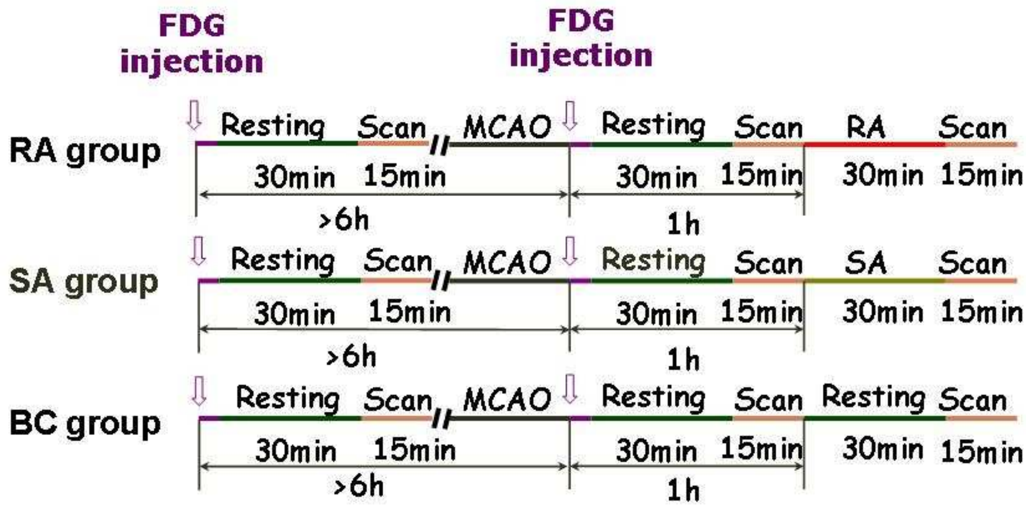

Figure 2: An overview of the experiment flow chart. The image data were acquired three times for each rat: baseline scanning; scanning right after MCAO; and scanning after real acupuncture/sham acupoint treatment/resting (for RA/SA/BC group).

Procedure

We followed the standard animal experimental procedure in our study. All rats were housed in the animal care facility with free access to food and water before and after the experiments. The study was approved by the Animal Research Committee of Zhejiang University, School of Medicine, and the experimental procedures were carried out in accordance with the guidelines for the care and use of Laboratory Animals published by National Institutes of Health (NIH) of the United States. During the experiment, each rat was weighted and anesthetized with 400 mg/kg chloral hydrate twice: Once before the first MicroPET scanning and the other time before MCAO. The chloral hydrate was supplemented as necessary during the experiment. Figure 2 illustrates the experiment flow chart with the following details:

1. **Baseline FDG-MicroPET Scanning.** For each rat in the study, we acquired 3-D FDG-microPET images with an microPET R4 system (CTI Concorde Microsystems, LLC.) at the Medical PET Center of Zhejiang University. The microPET scanner consisted of 32 detector rings with an animal aperture of 120 mm and an axial field-of-view of 78 mm. The spatial resolution in the scanner center was 1.88 mm FWHM in the axial plane, 1.9 mm FWHM in the transversal plane. Each rat

was positioned in the microPET R4 scanner in transaxial position with its head in the field of view. Concretely, we injected intravenously $0.5 \sim 1$ mCi FDG into the tail vein of the rat and put it back to the home cage in a quiet room for 30 minutes for glucose uptake. Then, we performed 3-D microPET scanning for 15 minutes. After that, we waited for at least six hours to let the FDG attenuate for more than three half lives.

2. **Animal Modeling.** We induced the focal cerebral ischemia by carrying out the commonly-used middle cerebral artery occlusion (MCAO) procedure (Bederson et al. (1986)) with craniotomy and electrocoagulation (Tamura et al. (1981)). The zygomatic and squamosal bones in the right head were exposed by a 1.5 cm incision in the skin, and the muscle over the bones was excarnated. A small hole of 2 mm in diameter was drilled in the junction part of the zygomatic and squamosal bones with a high speed hand-held drill. The right MCA was exposed by retracting the meninges with ophthalmic scissors and hemostatic forceps and then permanently occluded using bipolar electrical coagulation. Disinfected absorbent cotton was employed to fill the surgical field, and the skin was closed with suture.

3. **Resting and MicroPET Scanning.** Right after the MCAO step, we injected FDG again. Then, we followed the same procedure of resting for 30 minutes and scanning for 15 minutes as described in step 1. To assure synchronization, we moved to step 4 at the one hour time-point after the second FDG injection.

4. **Real Acupuncture, Sham Acupoint Treatment and Blank Control.**

   – For each rat in the RA group, we carried out real acupuncture for 30 minutes by following the common guideline of clinical practice: Stainless filiform needles (0.3 mm in diameter and 13.0 mm in length) were inserted into the acupuncture points for $5.0 \sim 10.0$ mm deep for Baihui and for $2.0 \sim 3.0$ mm deep for Shuigou, respectively. The needles were manually twisted for one minute at a frequency of about 2Hz and then left still for four minutes. This pattern is repeated for six times during the manipulation session.

After 30 minutes, we removed the needles and then carried out the third microPET scanning.

– For each rat in the SA group, we carried out sham acupoint treatment for 30 minutes. Then, we removed the needles and carried out the third microPET scanning. The needle manipulation follows the same pattern as that in the RA group.

– For each rat in the BC group, we kept it in its home cage under the resting state for 30 minutes and then carried out the third microPET scanning.

In the above procedure, we ignored the timing effect (or nature recovery effect) by scanning the rats in the acute stroke phase. Also, we chose the simplest combination of Baihui and Shuigou to avoid over-complication in acupoint combination. The pros and cons of this design will be discussed in more details in the discussion section.

Furthermore, some people suspected that the electric current effect in electroacupuncture is more dominant than the acupuncture effect. Our design bypassed this issue by only using manual acupuncture and following common pattern in clinical practice. To avoid human bias and individual variation, a single well-trained acupuncturist did all the acupuncture throughout the entire study.

After microPET scanning, 3-D microPET images were reconstructed using a maximum a posteriori (MAP) algorithm (Kornblum et al. (2000)). Figure 3 shows one example of the rat brain after MCAO modeling. The injury-caused glucose metabolic decrease is visible in the front lobe (upper-right area) of the brain.

Region of Interests

In order to verify the location of injured area in the brain induced by MCAO, we also carried out two other morphological measurements: Magnetic resonance imaging (MRI) and triphenyl tetrazolium chloride (TTC)-staining (Chiamulera et al. (1993)). We acquired MRI images and diffusion weighted MRI images for 15 modeled rats about 12 hours after MCAO and did TTC-staining for 14 modeled rats about 24 hours after MCAO. One MRI example and one TTC example are shown in Figure 4 and Figure 5, respectively. Please note that they are not from the same rat. All examples are available at http://bioinformatics.ust.hk/acupuncture.htm.

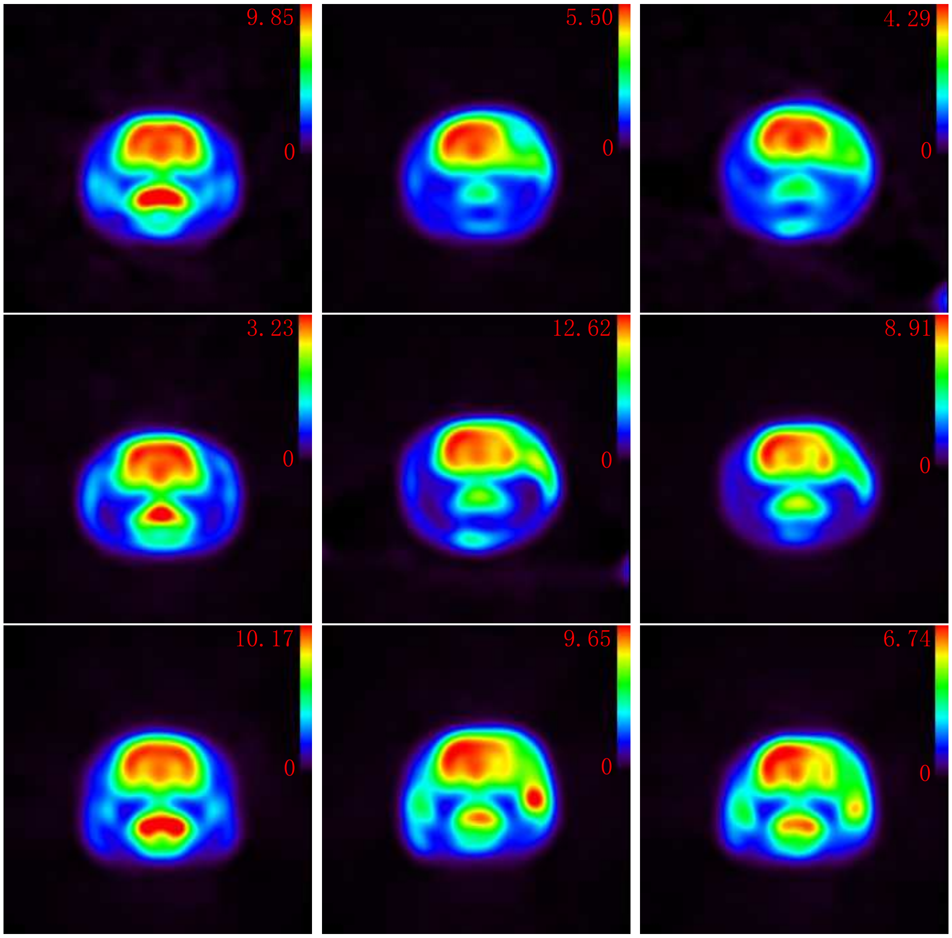

Figure 3: FDG-microPET image examples of representative coronal sections. The right hemisphere of the rat brain (shown as right-hand side in each figure) is the area where ischemia injury happens. **Top to bottom:** RA group example (ID 0001) , SA group example (ID 0011), and BC group example (ID 0009). **Left to right:** Baseline scan, post-MCAO scan, and final scan. Full volume images of these examples and other images are available at http://bioinformatics.ust.hk/acupuncture.htm. Here, the activity images are reconstructed by using the MAP algorithm (Kornblum et al. (2000)). Metabolic activity after real acupuncture increases significantly in the injured area; there was no evidently metabolic recovery after sham acupoint treatment; a metabolic deterioration was observed in the blank control case. Note that different images have different ranges of intensity values. This is unavoidable since we have different FDG-concentrations in different rats during micro-PET imaging due to the fact that the FDG-reagent had to be produced on a daily basis and be used at different time points. This variation vividly explains the reason (in addition to individual difference among different rats) that we used relative index instead of absolute intensity values in function comparison.

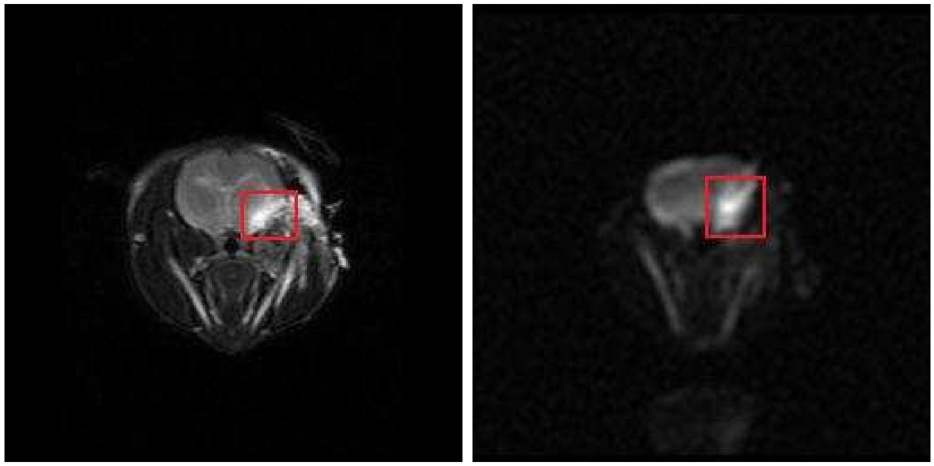

Figure 4: One selected slice with the coronal view of the MRI image (left) and that of the corresponding diffusion weighted MRI image (right) from the rat with ID PA9. The red rectangle in the image indicates the injured cerebral area.

These morphological measurements confirmed that the injured area was indeed in the right hemisphere of the cerebral cortex, as previously reported in (Engelhorn et al. (1999)). This confirmation largely simplified our analysis of images as we could ignore irrelevant regions in the brain.

In order to minimize the location variation and size variation of different rats, we aligned different rat brains together by using the software Statistical Parametric Mapping (SPM) (Friston et al. (1995)) with the default 12-parameter affine transformation. Since SPM was developed for human brain mapping and rat brain size is much smaller than human brain size, we scaled up the voxel size by a factor of eight to use the default parameter settings in SPM.

The SPM software also provides voxel-wise statistical tests. Ideally, this should allow us to identify significant difference of brain functions at the voxel level. In our analysis, however, we found that the results of such an analysis were not stable. One reason was that perfect alignment was not readily achievable for small animal studies. Another reason might be that the locations of MCAO may change for different rats due to experimental variations. Consequently, the affected area may not be very consistent at the voxel level, causing instable results of voxel-wise analysis.

As a remedy, we drew a three-dimensional spherical region of interest (ROI) with a fixed radius in the frontal lobe to locate the affected area

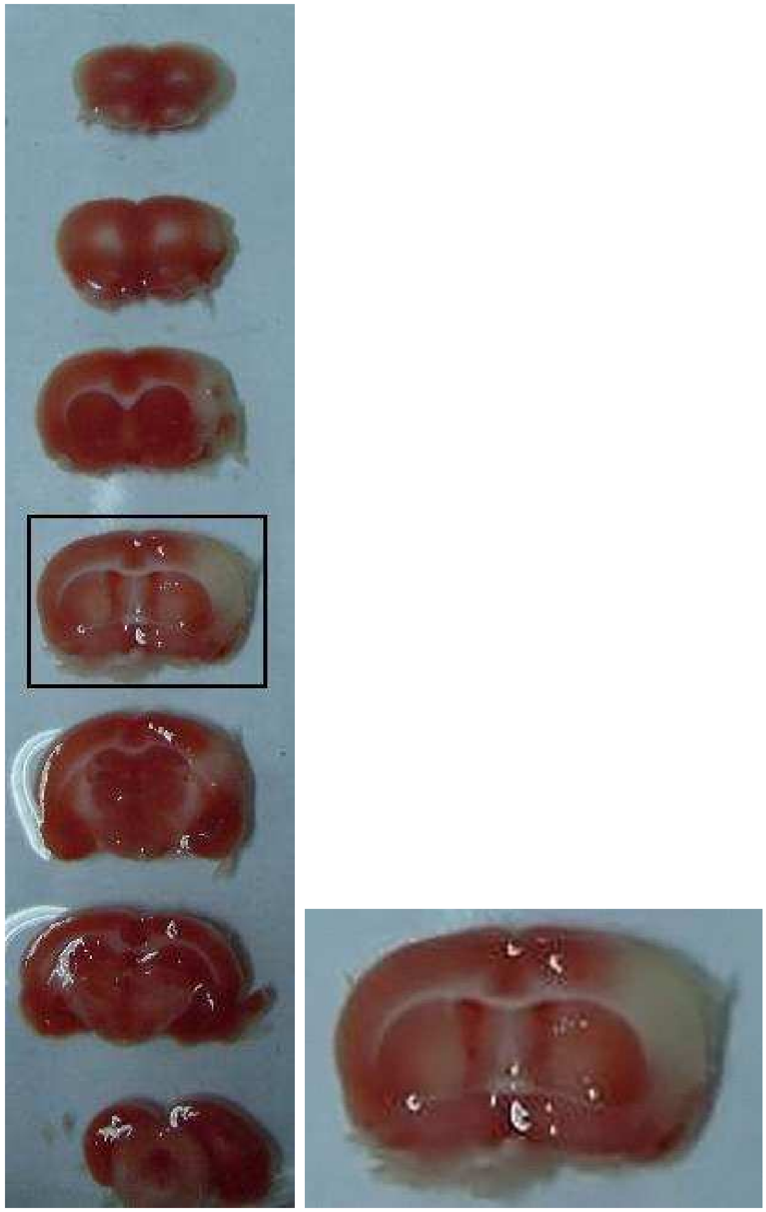

Figure 5: The TTC stained brain from the rat with ID SDC10226. **Left top to left bottom:** The coronal sections from rostral to caudal. The figure in the third row (black rectangle) is further zoomed-in on the right.

and then used the average intensity value in the ROI as the quantitative measure of brain function. Resolution-wise ROI-based analysis is worst than voxel-level test. But it will be more robust. It should be noted that reduced glucose consumption was observed in both cortex and sub-cortex of the brain. To simplify our analysis, we only chose the cortex area when drawing the spherical ROI.

Statistical Analysis

Directly using the average intensity value within the ROI cannot avoid the variations of FDG-concentration in different rats and imaging timing. Thus, we chose to use a relative index. There are different relative indices to measure the glucose consumption in the brain (Heiss and Herholz (1994)). In this paper, we used the following index to measure the relative glucose metabolic change (Heiss and Herholz (1994)):

$$CMR = I_r/I_l \times 100$$

with I_l and I_r denoting the average intensity value in the left ROI and right ROI, respectively. Here we assumed that the glucose metabolic levels in the left and right hemispheres were equal before MCAO (i.e., $CMR = 100$ for normal cases). After MCAO, we only considered those rats with $CMR < 90$ in our analysis. This step helped to remove imperfect cases of MCAO and only kept those rats with significant ischemia.

In our experiments, we have done MCAO on 253 rats. After applying this criterion, only 110 rats in our study (40 rats from the RA group, 54 rats from the SA group, and 16 rats from the BC group) remain. We discarded a large number of rats because MCAO is a highly technical procedure and the consistency of MCAO is critical for us to build a relatively homogeneous animal disease model. Fortunately, we still had enough number of rats remaining to draw statistical conclusions.

As the severity of ischemia varied from rat to rat, we used the following index (RI) to measure the recovery of the metabolic change (Martinez-Vila et al. (1990)):

$$RI = (CMR_3 - CMR_2) \times 100/(100 - CMR_2)$$

with CMR_2 and CMR_3 denoting the relative glucose metabolic change in the second and the third microPET image, respectively.

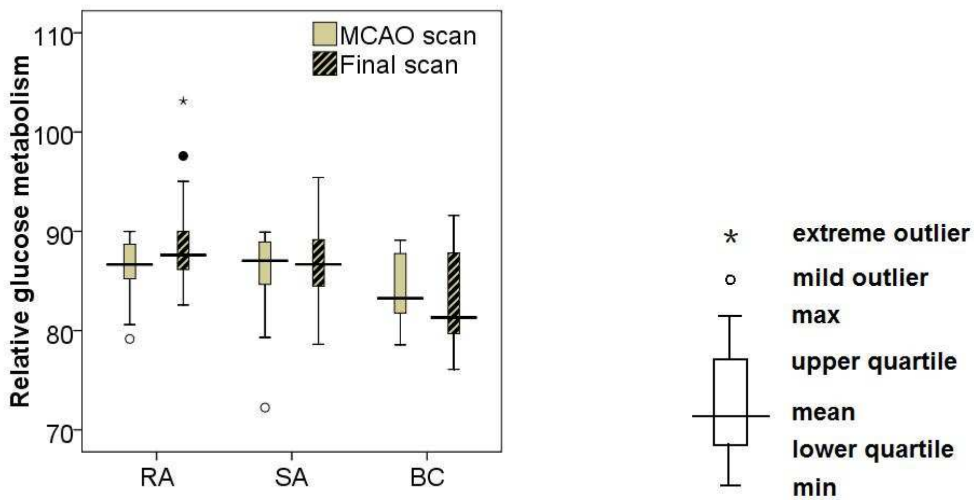

Figure 6: **Left:** Box plots of CMR values from three groups after MCAO and the final scan. **Right:** Explanation of the box plot.

Table 1: Pair-wise t-test and permutation test results of three groups. We assumed equal variance during the t-test. This assumption was verified with F-test.

	RA vs. SA	RA vs. BC	SA vs. BC
P-Value in t-test	0.0232	0.0221	0.1554
P-Value in permutation test	0.0244	0.0162	0.1589

Results

For the resulting 110 RI values from three groups, Figure 6 gave a box plot to describe their distributions. The metabolic recovery after real acupuncture was shown to be better than those after sham acupoint treatment and blank control. To verify if the differences of their mean values are statistically significant, we also carried out the t-test and permutation test. The P-values in Table 1 showed that the RA group provided a significantly higher mean RI value than the other two groups, while the mean difference between the SA group and the BC group was insignificant. This result strongly supports that acupuncture is more effective than random stimulus in improving the metabolic recovery after stroke.

Discussion

According to the TCM theory, acupuncture at Baihui and Shuigou is among the first choices in treating lesions in the brain. As the glucose metabolic level is a quantitative measure of the brain function, the experiments demonstrated the positive correlation between our measurement and TCM prediction. In this sense, our experiments have achieved the goal of positive verification. It remains unclear, however, how the acupuncture stimulates the increase of glucose. One manipulation may be that the neural system reacts to acupuncture more actively and regulates the increase of blood flow and the glucose metabolism. But more carefully designed studies are needed to test such a hypothesis. For example, we also need to study the effect of acupuncture on brain glucose consumption of resting rats (i.e. without MCAO modeling). This could be very interesting for us to further understand the effect of acupuncture.

In our experiments, we have chosen certain specific settings to simplify the experiments and the corresponding interpretation. Here we like to discuss about the pros and cons of our choices.

- Acupoint combination is a complicated issue. Among different acupoints, Baihui (GV20) is the most important one in treating ischemic stroke and it is easy to locate. Considering that Shuigou is also easy to locate and the combination of Baihui and Shuigou is commonly used in clinics for patient treatment, we chose such a combination in our experiments. While this choice may not be the most effective one in terms of treating stroke, our goal here was to verify the effectiveness of acupuncture instead of finding the most effective acupoint combination.

- In the experiments, we chose the sham acupoints to be 5mm lateral to Baihui and 5mm lateral to Shuigou. The first sham acupoint is close to the acupoint of Sishencong (EX-HN1), which also has some effect on treating stroke according to TCM theory, while the second sham acupoint is close to the acupoint of Kouheiao (LI19). One concern is that we may use true acupoints as sham acupoints due to variations in positioning the needles, thus jeopardizing the real contrast between true acupoints and sham acupoints.

  According to the rat acupoint atlas (Hua et al. (1991); Li (2003)), many acupoints are located in the head, making it difficult to choose sham acupoints that are far enough from some other acupoints. In our experimental design, our intension was to keep enough distance between sham acupoints and Baihui/Shuigou. We were aware of the closeness between sham acupoints and other acupoints. But it should be noted that Sishencong along with Kouheiao should have weaker effect in ischemic stroke treatment according to TCM theory. Even when we used these alternative acupoints as the shame acupoints due to experimental variation, the more positive effect of Baihui and Shuigou combination has also been verified. In this sense, our finding is still consistent with the TCM theory.

- In our experiments, we only considered the glucose metabolic level at around one hour after MCAO modeling and ignored the timing effect (or nature recovery effect) of stroke. We made such a choice to carry out a well-controlled study. For example, we did not need to worry about other pathological complications in the chronic recovery phase after the stroke (Young et al. (1997)). On the one hand, such a choice significantly simplified the experimental procedure and the interpretation of experimental results. On the other hand, this choice excluded our chance of observing dynamic responses of test animals during the acute and sub-acute stages of ischemia stroke and long-term effect of acupuncture. In our future work, we plan to extend the observation window by having multiple PET scanning of the same test animal after MCAO. This extension will help us to better understand the dynamic changes of metabolic activities.

Conclusion

In this paper, we used the glucose metabolic level as a quantitative index to measure the brain activity. By comparing real acupuncture with sham acupoint treatment and blank control under a simplified animal experiment setting, we were able to verify that acupuncture indeed increased the glucose metabolic level in the acute stage of ischemia stroke. While this finding is still far from thoroughly verifying the effectiveness of acupuncture in stroke treatment, it showed that we can probe the consequences of acupuncture quantitatively with target-specific imaging technique. As acupuncture and traditional Chinese medicine in general shared the reputation of being fuzzy, our approach provided a feasible example of explaining concepts in traditional Chinese medicine with modern scientific language.

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