Research Article

# Acupuncture enhances brain function in patients with mild cognitive impairment: evidence from a functional-near infrared spectroscopy study

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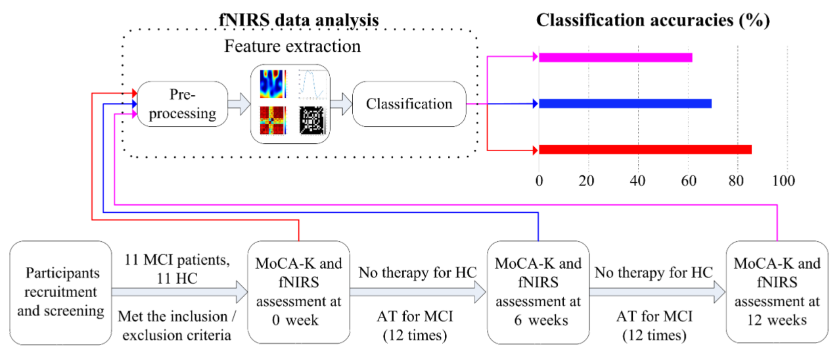
**Graphical Abstract** *Investigating the effectiveness of longitudinal acupuncture therapy on mild cognitive impairment (MCI) patients through classification*




## Abstract

Mild cognitive impairment (MCI) is a precursor to Alzheimer's disease. It is imperative to develop a proper treatment for this neurological disease in the aging society. This observational study investigated the effects of acupuncture therapy on MCI patients. Eleven healthy individuals and eleven MCI patients were recruited for this study. Oxy- and deoxy-hemoglobin signals in the prefrontal cortex during working-memory tasks were monitored using functional near-infrared spectroscopy. Before acupuncture treatment, working-memory experiments were conducted for healthy control (HC) and MCI groups (MCI-0), followed by 24 sessions of acupuncture for the MCI group. The acupuncture sessions were initially carried out for 6 weeks (two sessions per week), after which experiments were performed again on the MCI group (MCI-1). This was followed by another set of acupuncture sessions that also lasted for 6 weeks, after which the experiments were repeated on the MCI group (MCI-2). Statistical analyses of the signals and classifications based on activation maps as well as temporal features were performed. The highest classification accuracies obtained using binary connectivity maps were 85.7% HC *vs*. MCI-0, 69.5% HC *vs*. MCI-1, and 61.69% HC *vs*. MCI-2. The classification accuracies using the temporal features mean from 5 seconds to 28 seconds and maximum (i.e, max(5:28 seconds)) values were 60.6% HC *vs*. MCI-0, 56.9% HC *vs*. MCI-1, and 56.4% HC *vs*. MCI-2. The results reveal that there was a change in the temporal characteristics of the hemodynamic response of MCI patients due to acupuncture. This was reflected by a reduction in the classification accuracy after the therapy, indicating that the patients' brain responses improved and became comparable to those of healthy subjects. A similar trend was reflected in the classification using the image feature. These results indicate that acupuncture can be used for the treatment of MCI patients.
**Key Words:** acupuncture; Alzheimer's disease; cognition; convolutional neural network; functional connectivity; functional-near infrared spectroscopy; hemodynamic response; linear discriminant analysis; mild cognitive impairment

## Introduction

Neurological injuries and diseases are the major causes of the degradation of people's mental capabilities that can be treated via rehabilitation. One of the brain diseases is Alzheimer's disease (AD), which starts with mild memory loss and eventually becomes severe (Si et al., 2019). According to a study, 70% of dementia cases are caused by AD worldwide (Alzheimer's Association, 2019). Mild cognitive impairment (MCI) is a condition that occurs as a prior stage of AD. It is defined by a loss of vocabulary and judgment-related memory. It progresses rapidly, which can lead to AD. Therefore, early diagnosis and treatment of MCI—involving either therapy (acupuncture) or medication-based care—is necessary. Acupuncture is a non-pharmacological procedure based on the placement of tiny needles at particular locations on the human body, known as acupoints (Lee et al., 2020). The acupuncture-related changes in the brain are still being investigated; however, it has neurological outcomes such as improvement in cognition and stroke treatment (Li et al., 2020).

AD/MCI pathogenesis can be studied using electroencephalography, functional near-infrared spectroscopy (fNIRS), and functional magnetic resonance imaging (fMRI) (Khan et al., 2018; Hong and Yaqub, 2019). For more than a decade, fMRI has been employed to study the underlining neural mechanisms elicited through acupuncture

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(Asghar et al., 2010; Bai et al., 2010; Hui et al., 2010; Liu et al., 2011; Chen et al., 2012; Feng et al., 2012). Many studies have utilized fNIRS for showing the changes that occur in the hemodynamic responses of MCI patients during the task. Arai et al. (2006) found that the HR of MCI patients was smaller in the parietal region as compared with healthy controls (HC). Yeung et al. (2016a, b) showed loss of lateralization in MCI patients and observed no increased activation during a cognitive task. Decreased activation in the prefrontal cortex and parietal areas of MCI and AD patients was observed in similar seminal works (Uemura et al., 2016; Li et al., 2018). Improved cortical activation was observed in a dual-task (walking and verbal frequency task), and reduced activation was observed during normal walking in MCI patients (Doi et al., 2013). Yap et al. (2017) revealed that the time taken to achieve the activation level of maximum HR in the prefrontal cortex of HC was shorter than that of MCI or AD patients. These results showed the potential of fNIRS for the detection of MCI/AD. However, the influence of acupuncture on MCI patients using fNIRS is still in the developing stage. The preceding study had investigated the longitudinal effects of acupuncture on MCI patients: The analyses were primarily based on the Korean version of the Montreal Cognitive Assessment score (MoCA-K) and HRs (Ghafoor et al., 2019). The improvement in mean MoCA-K scores and averaged HRs depicted that the cognitive function of the MCI group was enhanced upon acupuncture. Nevertheless, the conclusion on the averaged increase in fNIRS data and MoCA-K scores was deduced based on statistical analyses.

To validate the impact of acupuncture, we aim to explore the changes at the trial-level (not averaged) temporal data, spatial activation patterns, and functional connectivity by employing classification algorithms. We aimed to determine whether acupuncture can improve the mental health of MCI patients in terms of brain activation. We attempted to determine whether there are changes in the HRs of the patients due to acupuncture concerning the classification accuracies. To check our hypothesis, two classification methods were adopted in the study, i.e., classification using temporal features and through a convolutional neural network (CNN).

## Subjects and Methods

### Ethical approval

The study protocol was approved by the Institutional Review Board of Dunsan Korean Medicine Hospital, Daejeon University (IRB No. DJDSKH-17-BM-13) on June 5, 2017 (**Additional file 1**), and conducted in accordance with the *Declaration of Helsinki*. This study was reported according to the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement (von Elm et al., 2014). Before the experiment, all the participants were given a complete description of the experimental procedure and provided written informed consent. For the MCI patients, additional consent was obtained for the acupuncture. The Clinical Research Information Service (CRIS) of the Republic of Korea (https://cris.nih.go.kr/cris/en/), which is a publically accessible primary register that participates in the WHO International Clinical Trial Registry Platform, approved this trial having registration number KCT 0002451, registered on September 5, 2017.

### Participants

In this case-control observational study, eleven HC and eleven MCI patients were recruited. For consistency, the average age, sex, and number of years of education were comparable between the groups. A total of 11 healthy (age 55.92 ± 7.65 years) and 11 MCI patients (age 61.58 ± 6.55 years) were recruited in the experiment (*P*-value for group differences = 0.0755). To avoid gender variability, all the participants were female. MCI patients were recruited from Dunsan Korean Medical Hospital in the year 2017 (September–December). Newspapers were also used for advertisement to recruit participants. All the participants were informed that they could stop participating at any time. The medical history of the participants, along with demographic and drug-intake information, was obtained before the experiment. Additionally, on-spot assessments of the height, weight, and systolic blood pressure were performed. None of the participants had indulged in a brain signal acquisition experiment before this study. To evaluate the cognitive performance of the participants, the MoCA-K test was performed. The inclusion criteria for HC and MCI patients were: (i) The ages range from 40 to 80 years; and (ii) MoCA-K scores are < 22 for MCI and > 26 for HC. The exclusion criteria were unstable psychiatric disorders and/or the presence of other neurological injuries/illnesses such as Parkinson's disease, stroke, cerebral hemorrhage, tumors, and schizophrenia.

The needed number of subjects was calculated using statistical basis/power analysis. We performed the power calculation by a two-sided test with a 5% level of significance, with the statistical power to be 80% for computation of the sample size. We planned to conduct a two-arm clinical trial to evaluate the effects of acupuncture therapy, which may enhance the cognitive ability of MCI patients. Our hypothesis was the increase of 6 units in the MoCA-K test scores of MCI patients after acupuncture therapy, which would represent a clinically meaningful increase.

The effect size was calculated using an estimated value of the variability in the MoCA-K test. A two-sided test was used with a 5% level of significance $Z$ (1–$\alpha$/2) = $Z$ (0.975) = 1.960 and the power of the statistical test to be 80% $Z$ (0.8) = 0.84 to compute the sample size that comes out to be 10. The anticipated dropout rate was set to 15% because it was expected that some participants might not be able to complete the study. Therefore, the required number of subjects in each group will be 10/0.85 = 12. During the experiment, one MCI patient's fNIRS data were not recorded properly: This result was not included in the subsequent analysis. Moreover, for a fair comparison, one healthy control subject's data were also excluded in further analysis.

### Optode placement for fNIRS signal acquisition and experimental paradigm

In the experiment, seven detectors and eight emitters were positioned over the region of interest. **Figure 1** shows the optode configuration on the prefrontal cortex of the human brain. A total of 20 channels were formed using all the emitters and detectors. The optodes were placed by taking FpZ as a reference point. The reference points were set in accordance with the International 10–20 System of Electrode Placement. The separation between the emitter and detectors was ≤ 3 cm.

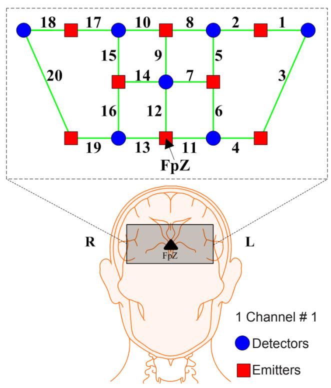


**Figure 1 | Emitter-detector configuration on the prefrontal cortex.**
FpZ is taken as a reference point in accordance with the International 10-20 System (Ghafoor et al., 2019).

For the prefrontal cortex, a working-memory task was employed to detect neuronal activation (Di Rosa et al., 2019). Nine trials were conducted during the experiment. Each trial consisted of a 24-second task period and a 14-second rest period. A "ready" cue of 2 seconds was given in each trial before the start of the task period. This was done to alert the subject that a task was to be performed soon. During the task period, for the initial 8 seconds, 3–7 images randomly appeared on a screen placed in front of the subjects. After the 8 seconds, there was a period of 14 seconds during which the subjects were allowed to hold images that they selected (as per difficulty). This was followed by a probe period of 2 seconds in which the participants had to respond, indicating whether the image displayed was among the images shown previously. In responding, the subjects used two push buttons to reply "yes" or "no." Before the commencement of the experiment session, there was a pre-rest period of 4 minutes. Similarly, at the end of the experiment, there was a 30-second post-rest period. **Figure 2** shows the experimental paradigm (Ghafoor et al., 2019). A black screen was displayed during the rest period. Throughout the experiment, the subjects were advised to keep their eyes open.

### Acupuncture

The acupuncture intervention details were presented in detail elsewhere (Jung et al., 2018). Briefly, the patients underwent several acupuncture sessions lasting 10 min each. A total of 24 sessions were conducted over 12 weeks (i.e., for brevity, before acupuncture: MCI-0, after 12 acupuncture sessions (6 weeks): MCI-1, and after 24

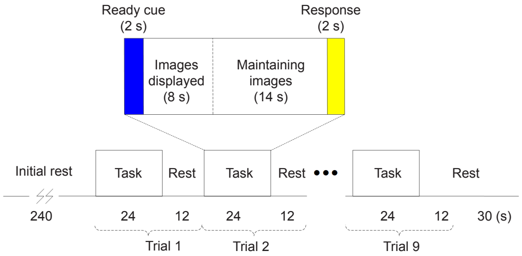


**Figure 2** | **Experimental paradigm.**

acupuncture sessions (12 weeks): MCI-2). The selection of acupoints was based on the 12-meridian system used in clinical trials for AD and MCI (Zhou and Jin, 2008; Chen et al., 2014; Liu et al., 2014; Shi et al., 2015). During this time, the participants were instructed not to participate in any other acupuncture. The acupuncture was performed under the rules of Standards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA) (MacPherson et al., 2010). **Table 1** summarizes the intervention details.

**Table 1** | **Acupuncture intervention details**

| Elements | Description |
|---|---|
| Acupuncture type | Traditional Korean medicine therapy |
| Number of needle insertion per subject and session | 14 acupoints |
| Location of acupoints | GV20, EX-HN1, CV12, (Shenmen HT7 bilateral), ST36 (bilateral), SP6 (bilateral), and Taixi (KI3 bilateral) |
| Penetration of needle | Depending upon the thickness of skin (approximately 5–10 mm) |
| Stimulation on needle insertion | No |
| Needle retention time | 10 min |
| Dimensions and material of needle | 0.20 mm × 30 mm in size, made of stainless steel (Dongbang Medical Co., Ltd., Korea) |
| Total number of acupuncture therapy sessions | 24 |
| Qualification of acupuncturists | A medical doctor with clinical experience of more than 2 yr |

### Acquisition and preprocessing of fNIRS signals

The signals generated in the brain were sampled at a frequency of 7.81 Hz. A single-phase continuous-wave fNIRS system NIRScout (NIRx Medical Technologies, New York, NY, USA) was used for the acquisition of fNIRS signals. The experiments were performed by the medical doctors and staff in Dunsan Korean Medicine Hospital, Daejeon University. Subsequently, the data analyses were done by the researchers in Pusan National University, Republic of Korea. Two different wavelengths were utilized by the system: 760 and 850 nm. The Modified Beer-Lambert's law was used to convert the raw intensities into changes in oxy-hemoglobin (ΔHbO) and deoxy-hemoglobin (ΔHbR) (Kocsis et al., 2006). This conversion was performed using the NIRSlab software (NIRx Medical Technologies).

After the data were converted into ΔHbO and ΔHbR, they were preprocessed to remove the effects of physiological noises. For this, a $4^{th}$-order Butterworth filter was utilized. A low-pass cutoff frequency of 0.15 Hz was used to remove cardiac, respiratory, and low-frequency drift signals (Fekete et al., 2011; Kainerstorfer et al., 2015). The cutoff frequency for the high-pass filter was selected according to the longest period of a single trial (i.e., 38 seconds (1/38 seconds = 0.026 Hz)) (Pinti et al., 2018; Zafar and Hong, 2018). For analysis, the software MATLAB 2017 was used (MathWorks, Naticks, MA, USA).

### Data analysis

After the pre-processing, the next step was to classify the data. From the literature, different sets of features can be found that have been utilized for data analysis (Noori et al., 2017; Nazeer et al., 2020). For classification, two different methods were used: Classification based on temporal features and classification using images (Yang et al., 2019). For classification based on temporal features, mean (i.e, mean(5:28 seconds) for brevity), maximum (i.e, max(5:28 seconds)), slope (i.e, slope(5:28 seconds)), skewness (i.e, skew(5:28 seconds)), kurtosis (i.e, kurt(5:28 s)), increasing slope (i.e, slope(2:12 seconds)), decreasing slope (i.e, slope(26:36 seconds)) and entire mean (i.e, mean(1:38 seconds)) were used as features. These features were classified using linear discriminant analysis (Naseer et al., 2016). Fivefold cross-validation was performed to determine the classification accuracy.

For analysis using images, the data for all the trials were first converted into pictorial form. Activation maps and connectivity maps were used as image features. For the representation of the activation maps (i.e., *t*-maps), a *t*-test was performed. The *t*-values for all the channels were calculated using the built-in function robustfit of MATLAB. In the test, the statistical significance level was set as 0.05. To obtain connectivity matrices, the Pearson correlation coefficients were calculated for the data of each channel (Zhao et al., 2020). These connectivity matrices were then used to create connectivity maps. The obtained images were classified using CNN. In this study, the neural network consisted of two convolutional layers and two fully connected layers. Again, fivefold cross-validation was used. Additional details regarding the method used in this study and the advanced machine learning approach were presented in the previous works (Kim et al., 2019; Kim and Choi, 2019; Yang et al., 2019). For detecting cortical activation, the time-series signal for each trial was fitted to the desired hemodynamic response (dHRF) signal (Hong et al., 2017).

### Statistical analysis

To evaluate the statistical difference between the MCI group and the healthy group, Student's *t*-tests were performed using software MATLAB 2017 (MathWorks). While comparing the average amount of activation between the healthy subjects and MCI patients, two independent sample *t*-tests were performed. Whereas paired *t*-tests were used for the comparison of the activation between the MCI patients before and after acupuncture sessions. Data are expressed as the mean ± SD. A difference was considered to be statistically significant if the following two conditions were satisfied: i) *t*-value > critical *t*-value ($t_{crt}$) and ii) $P$-value < 0.01. To verify the results based on functional connectivity, independent sample and paired *t*-tests were performed. The values of correlation for both healthy and MCI groups were converted from *r*-values to *z*-values. This was done using Fisher's *r*-to-*z* transform.

## Results

### Comparison of hemodynamic responses

The hemodynamic response function (HRF) is a typical response that is detected for healthy subjects and patients. To describe the shape of the HRF, the dHRF was used to identify the activation in each trial. After the active trials were extracted for all the subjects, the trials were averaged. The same procedure was followed for both groups. Visual inspection revealed credible activation for both the patients and the healthy subjects. However, the responses of the MCI patients obtained before and after acupuncture sessions differed significantly. **Figure 3** shows the maximum amount of activation observed for the MCI and HC groups. The maximum amount of activation for HC was significantly higher compared with MCI-0 ($P$ < 0.001). Interestingly, after the first twelve acupuncture sessions, there was no longer a statistically significant difference in the responses between the HC and MCI-1 groups ($P$ = 0.377). However, statistically significant differences were observed when the data of the MCI-0 were compared with MCI-1 ($P$ < 0.001). The activation in the case of MCI-2 was lower than HC group ($P$ < 0.001).

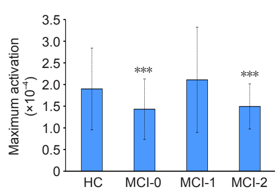


**Figure 3** | **Comparison of the maximum activations of oxy-hemoglobin over all the subjects.**

For both groups, the amounts of activation were represented using bars, and the standard deviations were represented as dotted lines above them. HC: Healthy control; MCI-0: before acupuncture; MCI-1: after 12 acupuncture sessions (6 weeks); MCI-2: after 24 acupuncture sessions (12 weeks). Data are expressed as the mean ± SD with $n$ = 11 HC and $n$ = 11 MCI. ***$P$ < 0.001, *vs*. HC. MCI: Mild cognitive impairment.

**Figure 4** shows the obtained average MoCA-K scores for both HC and MCI. To evaluate the task response of subjects, working memory adaption rates were calculated after each fNIRS experiment. Interestingly, statistically significant results were obtained while comparing the working memory adaption rate of HC and MCI-0 ($P$ = 0.004), but there was no statistical significance the results of HC were compared to MCI-1 and MCI-2 ($P$ = 0.033 and $P$ = 0.034, respectively).

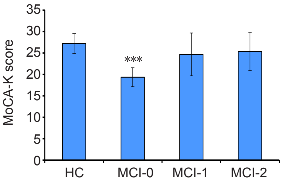


**Figure 4 | Comparison of the averaged MoCA-K scores in the participants.**
The standard deviations in the score are represented by lines in the figure. (a) HC, (b) MCI-0, (c) MCI-1, (d) MCI-2. HC: Healthy control; MCI-0: before acupuncture; MCI-1: after 12 acupuncture sessions (6 weeks); MCI-2: after 24 acupuncture sessions (12 weeks). Data are expressed as the mean ± SD with $n$ = 11 HC and $n$ = 11 MCI. ***$P$ = 0.004, *vs.* HC. MoCA-K: Montreal Cognitive Assessment score; MCI: mild cognitive impairment.

### Classification

For classification, all the features were extracted and then used in pairs. The classification accuracy obtained using each of these feature sets is shown in **Figure 5**. The maximum classification accuracy obtained using mean (i.e, mean(5:28 seconds)) and maximum (i.e, max(5:28 seconds)) values were 60.6% for HC *vs.* MCI-0, 55.9% for HC *vs*. MCI-1, and 56.4% for HC *vs*. MCI-2. According to the results, most of the feature combinations yielded a classification accuracy higher than the chance level (i.e., 50%).


**Figure 5 | Comparison of classification accuracies for different feature sets in the participants.**
Feature sets: mean (i.e, mean(5:28 seconds) for brevity), maximum (i.e, max(5:28 seconds)), slope (i.e, slope(5:28 seconds)), skewness (i.e, skew(5:28 seconds)), kurtosis (i.e, kurt(5:28 seconds)), increasing slope (i.e, slope(2:12 seconds)), decreasing slope (i.e, slope(26:36 seconds)) and entire mean (i.e, mean(1:38 seconds)). HC: Healthy control; MCI-0: before acupuncture; MCI-1: after 12 acupuncture sessions (6 weeks); MCI-2: after 24 acupuncture sessions (12 weeks). $n$ = 11 HC and $n$ = 11 MCI. MCI: Mild cognitive impairment.

A decrease in the classification accuracies (after acupuncture sessions) can be seen in almost all of the feature combinations. This shows that after acupuncture, the temporal characteristics of the hemodynamic response of MCI patients became similar to the healthy subjects.

*t*-maps and connectivity maps are widely used in both functional magnetic resonance imaging and fNIRS for indicating the areas of activation of the brain. In this study, these maps were used for classification. **Figure 6** shows the *t*-maps for each subject in HC and MCI groups.

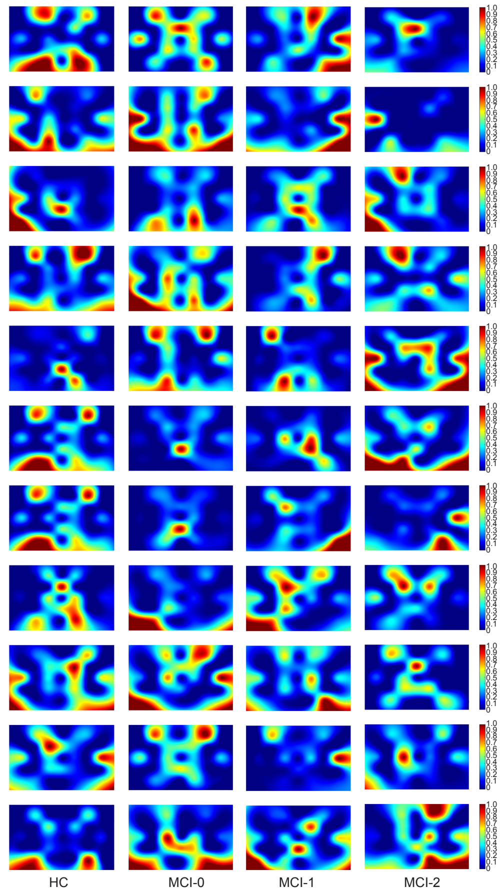


**Figure 6 | Activation maps of the brain generated using MATLAB for all participants.**
The brain activation has been shown through a color gradient from blue to red. The blue color means no activity whereas the red color shows maximum activation. $n$ = 11 HC and $n$ = 11 MCI. HC: Healthy control; MCI-0: before acupuncture; MCI-1: after 12 acupuncture sessions (6 weeks); MCI-2: after 24 acupuncture sessions (12 weeks). MCI: Mild cognitive impairment.

**Figure 7** shows the connectivity maps, whereas the corresponding binary matrices are shown in **Figure 8**. For converting the connectivity maps into binary matrices, a threshold value of 0.8 was used. The trend of the connectivity maps was similar to that of the t-maps. These results support the claim that acupuncture made the patients more normal. A significant difference was found while comparing the HC with MCI-0 groups ($P$ < 0.001). The same was the case for the comparison of functional connectivity maps of MCI-0 and MCI-1 (i.e., $P$ < 0.001). CNN was used for the classification of the images. **Figure 9** presents the classification accuracies obtained using *t*-maps. Whereas **Figures 10** and **11** show the classification accuracies obtained using connectivity maps and corresponding binary maps, respectively. As shown, the classification accuracies were changed due to acupuncture. After the therapy, the classification accuracy decreased, which is similar to the trend of the temporal features. However, among the three different types of maps used in the CNN-based classification, the binary matrices yielded the highest classification accuracy, i.e., 85.7% for HC *vs*. MCI-0, 69.5% for HC *vs*. MCI-1, and 61.69% for HC *vs*. MCI-2.

## Discussion

The objective of this study was to investigate the effects of acupuncture on MCI patients and to validate it as a method for improving their mental capabilities. It was hypothesized that acupuncture would change the hemodynamic response. Our second hypothesis was that acupuncture would change the activated area, resulting in making the patients respond like healthy subjects. To the best of the author’s knowledge, this was the first study in the field of fNIRS to investigate the effects of acupuncture concerning classification. We aimed to determine whether acupuncture can make patients normal by using fNIRS as a brain imaging tool. Our findings are summarized below.

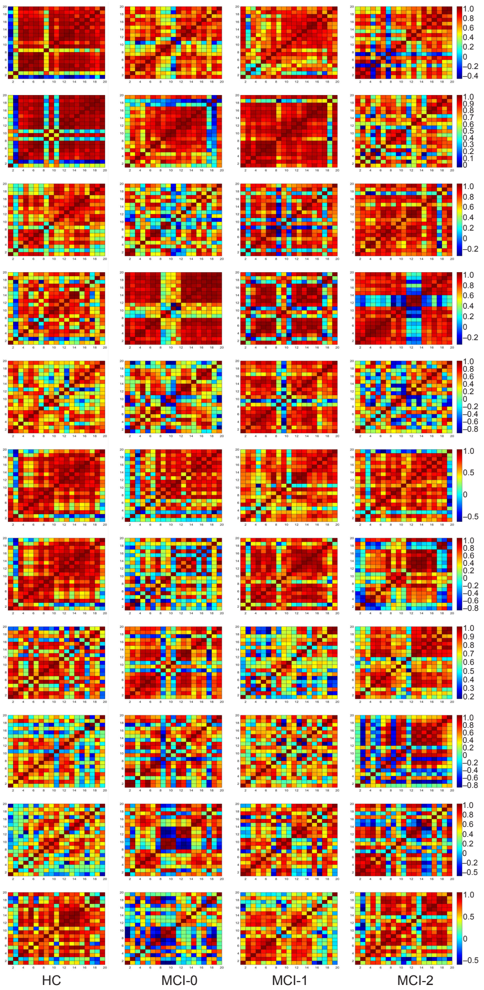


**Figure 7 | Connectivity maps of the brain generated using MATLAB software for all participants.**
The connectivity has been shown through a color gradient from blue to red. The blue color means no connectivity whereas the red color shows maximum connectivity. *n* = 11 HC and *n* = 11 MCI. HC: Healthy control; MCI-0: before acupuncture; MCI-1: after 12 acupuncture sessions (6 weeks); MCI-2: after 24 acupuncture sessions (12 weeks). MCI: Mild cognitive impairment.

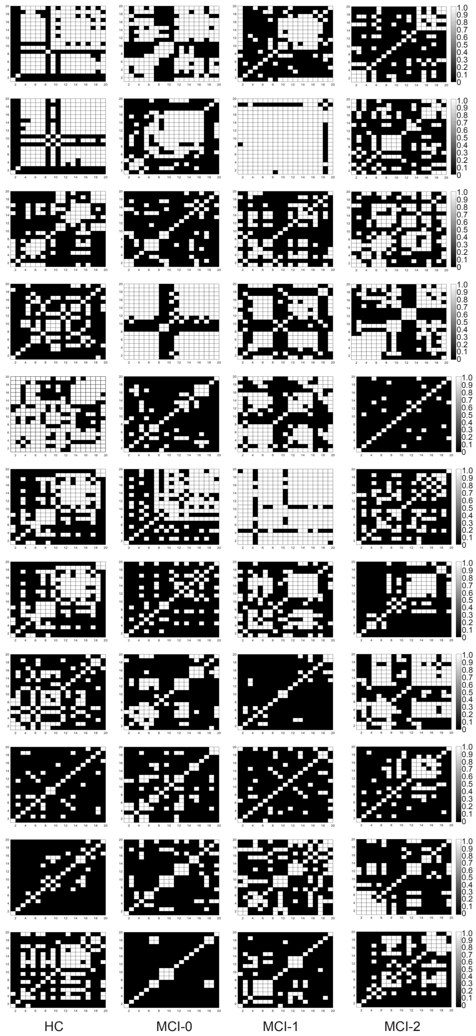


**Figure 8 | Binary matrices of all participants with the threshold of 0.8.**
HC: Healthy control; MCI-0: before acupuncture; MCI-1: after 12 acupuncture sessions (6 weeks); MCI-2: after 24 acupuncture sessions (12 weeks). *n* = 11 HC and *n* = 11 MCI. MCI: Mild cognitive impairment.

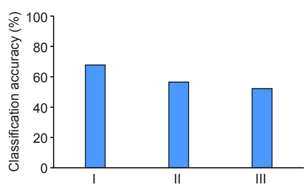


**Figure 9 | Comparison of convolutional neural network-based classification results obtained using t-maps.**
(I) HC *vs*. MCI-0, (II) HC *vs*. MCI-1, (III) HC *vs*. MCI-2. HC: Healthy control; MCI-0: before acupuncture; MCI-1: after 12 acupuncture sessions (6 weeks); MCI-2: after 24 acupuncture sessions (12 weeks). *n* = 11 HC and *n* = 11 MCI. MCI: Mild cognitive impairment.

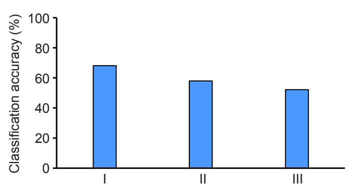


**Figure 10 | Comparison of convolutional neural network-based classification results obtained using connectivity maps.**
(I) HC *vs*. MCI-0, (II) HC *vs*. MCI-1, (III) HC *vs*. MCI-2. HC: Healthy control; MCI-0: before acupuncture; MCI-1: after 12 acupuncture sessions (6 weeks); MCI-2: after 24 acupuncture sessions (12 weeks). *n* = 11 HC and *n* = 11 MCI. MCI: Mild cognitive impairment.

i) As a first step, the brain activation area of the MCI group was compared with that of the healthy group. This was done both before and after the acupuncture. The brain activation patterns for the working-memory task were compared using a *t*-test. Initially, there were significant differences between the hemodynamic responses of the two groups. After 6 weeks, over which 12 acupuncture sessions were conducted, interestingly, there was no significant difference between the hemodynamic responses of the MCI and healthy groups. Thus, the statistical test validated our hypothesis, i.e., acupuncture made the patients more normal.

ii) For classification using temporal features, a feature set consisting of eight features was extracted from the hemodynamic responses of both groups. These features were then used as pairs to classify the healthy and MCI groups. Linear discriminant analysis was used for classification. The combination of the mean (5:28 seconds) and max(5:28 seconds) features yielded the highest classification accuracies. According to the results, in most of the cases, the classification accuracy was higher before the acupuncture than after it. Validation based on temporal features was in accordance with our hypothesis and the results of the statistical test. The results obtained through classification are in line with MoCA-K scores. It has been reported in the literature that behavioral results were improved due to physical acupuncture rather than sham acupuncture (Xu et al., 2020). In a future study, advanced control techniques and non-acupoints can be utilized to further validate the effects of acupuncture (Hong and Pham, 2019; Pamosoaji et al., 2019; Navabi et al., 2020; Rahmani and Rahman, 2020).

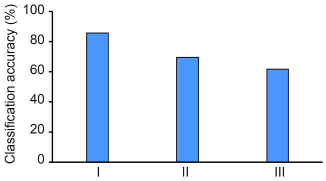


**Figure 11 | Comparison of convolutional neural network-based classification results obtained using binary maps.**
(I) HC *vs*. MCI-0, (II) HC *vs*. MCI-1, (III) HC *vs*. MCI-2. HC: Healthy control; MCI-0: before acupuncture; MCI-1: after 12 acupuncture sessions (6 weeks); MCI-2: after 24 acupuncture sessions (12 weeks). *n* = 11 HC and *n* = 11 MCI. MCI: Mild cognitive impairment.

iii) The prefrontal cortex of the brain is the major contributor to most active-memory tasks, such as mental arithmetic, counting, and working-memory tasks, as it is sensitive to mental training (Wang et al., 2012a). With age, this area becomes weak with regard to capabilities, giving rise to dangerous diseases such as AD (Sorg et al., 2007). Such changes in mental health can be monitored using brain activation maps and brain connectivity maps. Thus, the effects of acupuncture were validated using connectivity maps and activation maps.

Connectivity maps and activation maps were created for each trial. Using these maps, CNN-based classification was performed. For the MCI group, acupuncture increased the activated area and enhanced the connectivity in the prefrontal cortex. The results indicated that with the acupuncture, the activation areas and connectivity maps of the MCI patients became more similar to those of the healthy subjects. Additionally, the classification accuracy decreased, supporting the aforementioned hypothesis. These results indicate improvements in the patients and are in accordance with the literature (Serra et al., 2017; Ghafoor et al., 2019).

The foregoing results support our hypothesis that acupuncture makes MCI patients more normal with regard to brain health. The enhancement of the patients' mental state due to acupuncture is reflected by the ΔHbO signal, which is increased after acupuncture (Wang et al., 2012b; Yap et al., 2017). Although there is a decrease in the maximum value after MCI-2, the activation is higher as compared to MCI-0. Also, the improvement in the cognitive abilities of the MCI patients is reflected in the MoCA-K test scores. The results obtained through classification are in line with the predecessor of the current study (Ghafoor et al., 2019). Also, from the results of classification, there is a notable difference between the classification accuracies obtained from temporal features and image features. By using images of brain activation and connectivity, higher classification accuracies were obtained. These results are in line with a recently published work by (Yang et al., 2019). Overall, the results indicate that acupuncture is useful for enhancing the mental state of patients.

Owing to the small number of fNIRS channels available, the study was limited to the prefrontal cortex of the brain. In future research, a larger number of channels can be used for investigating other brain areas (Khan et al., 2020; Khan and Hong, 2021). Additionally, hybrid EEG-fNIRS neuroimaging modalities can be used for brain signal acquisition (Fu et al., 2017; Gong et al., 2017; Hong et al., 2020). This would be useful for verifying the hypothesis of the present study for the entire brain. A major limitation of fNIRS is inter-subject and intra-subject variability in the hemodynamic response signal (Sato et al., 2005). In this study, this has been catered for by taking overall data acquired from many subjects. Furthermore, it would allow observation of the activation and connectivity of the entire brain. To make the activation maps more precise, high-density or bundled optodes placement (including short separation channels) can be utilized to increase the spatial resolution (Nguyen and Hong, 2016; Yaqub et al., 2020). In future studies, Neuroplasticity needs to be checked several months later after the interventions to verify the long-lasting effects of acupuncture. Another limitation of this study was the number of subjects. Although the number of subjects was similar to those for previous studies, the study can be extended to a larger number of subjects. Moreover, only female participants participated in the study, representing another limitation, as the sex-based variability cannot be evaluated. In the future, identical research can be performed on male subjects, and the results can be compared with those of the present study. Another future endeavor will be to give acupuncture or electrical/magnetic stimulation only at the brain site for assessing their impact on different brain locations (Sabel et al., 2020). Evaluation of the immediate effect due to acupuncture in MCI patients and the different types of stimulation in acupuncture (i.e., by lifting, inserting, or twisting the needle) can also be a promising avenue in future studies (Rojas et al., 2019).

### Conclusion

This study is a step toward analyzing the effects of acupuncture treatment on MCI for enhancing the patient's brain activity. It was found that the traditional method of acupuncture can improve the mental health of patients, which was confirmed using classification methods. The results revealed that acupuncture therapy improved the hemodynamic responses of the patients. This claim is in line with the cognitive performance of the participants monitored through MoCA-K test scores. Additionally, the results indicated that the activated area of the MCI patients, as well as the connectivity, increased with acupuncture. Statistical evidence for these claims has been provided. Finally, the decrease in the classification accuracies indicated that the brain activation of the MCI patients became somewhat similar to those of the HC group due to acupuncture therapy.

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**Additional file:**
***Additional file 1:*** *Ethical Approval Documentation.*

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