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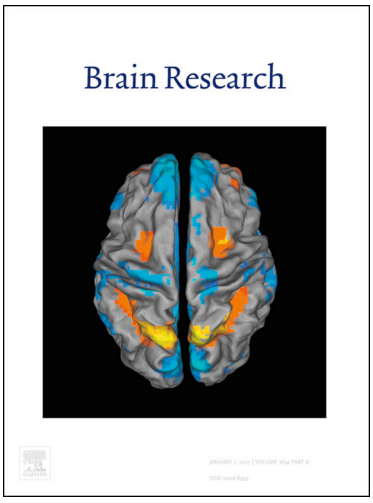

Cortical mapping of active and passive upper limb training in stroke patients and healthy people: A functional near-infrared spectroscopy study

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Cortical mapping of active and passive upper limb training in stroke patients and healthy people: A functional near-infrared spectroscopy study

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ABSTRACT

Active exercise for upper limb training has been widely used to improve hemiplegic upper limb function, and its effect may be boosted by extrinsic visual feedback. The passive movement of the hemiplegic upper limb is also commonly used. We conducted a functional near-infrared spectroscopy experiment to compare cortical activation during the following three conditions: active left upper limb movement (on the hemiplegic sides in stroke patients), with or without extrinsic motor performance visual feedback (LAV, LAnV), and passive left upper limb movement (hemiplegic sides in stroke patients) (LP) in stroke patients and healthy controls. Twenty patients with right hemispheric stroke and 20 healthy controls were recruited for this study. Hemodynamic changes were detected during left upper limb movements (on the hemiplegic sides in stroke patients) under the above three conditions in the sensorimotor cortex (SMC), supplementary motor area (SMA), and premotor cortex (PMC). There was no significant difference in the level of cortical activation between patients with stroke and healthy subjects during the three conditions. Both the LAV and LAnV induced significantly higher activation in the contralateral SMA and PMC than in the LP. Extrinsic visual feedback led to additional activation in the contralateral PMC and SMA, but this was not statistically significant. Our study indicates that active upper-limb movement appears to induce higher cortical activation than that elicited by passive movement in both stroke patients and the healthy population. Extrinsic motor performance in the form of visual feedback provided during active movement may facilitate sensorimotor areas over the contralateral hemisphere.

1. Introduction

Stroke is the second leading cause of death and third leading cause of disability-adjusted life years globally (World Health Organization, 2021). Most stroke patients experience physical

disabilities, and upper-limb motor dysfunction is a major concern (Kwakkel et al., 2015; Norouzi-Gheidari et al., 2012; Stephenson and Stephens, 2017). Motor impairment of the upper limb can result in life dependence, social isolation (Kwakkel et al., 2015; Small et al., 2013), and reduced quality of life (Nichols-Larsen et al., 2005). Therefore, it is necessary to restore the motor function of the upper limbs post-stroke.

Many interventions have been developed to improve upper-limb motor recovery, and active upper-limb motor training has been widely used in post-stroke upper-limb rehabilitation (Babaiasl et al., 2016; Langhorne et al., 2009). However, active motor training usually requires that participants have a certain level of upper-extremity motor function to achieve movements of the paretic arm; therefore, for patients who are weak or medically unstable, passive exercise can be an alternative choice. It has been hypothesized that reactivation of existing neuronal connections, development of new connections, and axonal regeneration in the neurosystem may contribute to the effectiveness of active and passive movement training in motor function recovery (Lindberg et al., 2004). Upper-limb motor training drives neuroplasticity in the sensorimotor cortex and, therefore, contributes to improvements in upper limb motor function in animal models (Nishibe et al., 2015). Studies on healthy subjects and stroke patients have shown that proprioceptive inflow during upper limb motor training activates the sensorimotor cortex (SMC), supplementary motor area (SMA), premotor cortex (PMC), and secondary sensory cortex, thereby improving motor performance (Carel et al., 2000; Lindberg et al., 2004; Yang et al., 2020). Furthermore, it has also been reported that abnormal brain activation in the sensorimotor area, usually hyperactivation in both hemispheres, when compared with healthy groups, has commonly been observed in people with chronic stroke during upper limb movements (Lim and Eng, 2019). Lim and Eng (2019) suggested that the stroke

population might experience cortical or peripheral inefficiencies and/or a greater sense of effort during the upper limb task, thereby causing greater cortical activation in the sensorimotor area. However, whether this overactivation recovers with improvement in upper limb motor function remains unclear.

The provision of extrinsic feedback may further facilitate cortical activation during active motor training (Subramanian et al., 2009; van Vliet, 2006). A previous study using functional near-infrared spectroscopy (fNIRS) examined cortical activation differences among stroke patients when performing three forms of lower limb cycling, and a higher activation was found in the PMC during active cycling with visual feedback on cycling speed than during active cycling without visual feedback or during passive cycling, whereas a similar cortical activation pattern was found in the latter two forms of cycling (Lin et al., 2013). The PMC has been proposed to be involved in controlling the speed of limb locomotion in stroke patients (Miyai et al., 2002). These findings suggest that activation of the PMC may play an important role when patients use extrinsic visual feedback to adjust their limb movements. It is still unknown whether different forms of upper limb movement (e.g., active movement with and without motor performance visual feedback and passive movement) lead to different changes in cortical activation in patients with stroke and in their healthy counterparts.

As a noninvasive neuroimaging technique, fNIRS has been utilized to investigate cortical activity by monitoring hemodynamic responses that result from neural activity during human locomotion. The advantages of portability and reduced sensitivity to motion artifacts with fNIRS have led to its widespread use in measuring cortical responses during motor tasks (Chen et al., 2021; Leff et al., 2011). In this study, we investigated cortical activation over the sensorimotor areas

during active upper limb movement with and without extrinsic motor performance visual feedback and during passive upper limb movement in stroke patients and healthy controls. We hypothesized that (1) cortical activation during upper limb movement will be greater in stroke patients than in healthy individuals, thus indicating greater movement efforts after a brain injury; (2) active upper limb movement will induce higher cortical activation than passive movement; and (3) extrinsic motor performance visual feedback would lead to additional cortical activation in the sensorimotor areas.

2. Results

Detailed information on 20 patients with left upper limb hemiparesis after a first-ever, right-hemispheric, subcortical stroke is shown in Table 1. The stroke group consisted of people diagnosed with first-time cerebral hemorrhage or infarction, with a mean (SD) disease duration of 223 (256) days. The upper limb motor function of stroke participants varied from level 3 to 7, as assessed by the Functional Test for the Hemiplegic Upper Extremity (FTHUE) (Fong et al., 2004). Patients with stroke at FTHUE levels 3 to 7 would have mild-to-moderate upper extremity impairment in their hemiplegic extremities. At level 3, stroke patients are able to move their hemiplegic upper limbs in a mass flexion pattern (shoulder joints at 30° to 60° and elbow joints at 60° to 100°) and are able to complete the arm clearance during shirt tuck and hold a pouch. Patients with higher levels of FTHUE had better motor function in the hemiplegic upper limbs.

According to the criterion, some deviant values from a healthy participant was detected in the passive left upper limb movement (LP) condition. The Cook's distances of these values are less than 1. Moreover, the results of statistical analysis with and without these outliers are identical (see

in Supplementary materials for the results of statistical analysis without outliers). No causal factor was identified during data collection as well. Therefore, these deviant values were not discarded from the following analysis. The results of the Shapiro–Wilk tests showed that most variables (approximately 80%) were normally distributed. Moreover, with samples of equal size greater than 5, the analysis of variance (ANOVA) is considered "robust" even when the assumptions of normality are not fulfilled, and homogeneity will not seriously affect the validity of the inferences drawn from the data (Norman, 2010; Portney and Watkins, 2014). Two-way ANOVAs were conducted, and Mauchly's test of sphericity was used to test the homogeneity of variance. If sphericity tests were violated, Greenhouse-Geisser epsilon adjustments were used.

The concentration changes of oxyhemoglobin (ΔHbO) values during the three conditions in patients with stroke and healthy controls are shown in Fig. 1. Supplementary Table 1 presents a two-way analysis of variance (ANOVA) by showing the main effects of the groups and conditions and their interaction effects on ΔHbO in the SMA, PMC, and SMC. A post hoc analysis is shown in Fig. 2.

2.1. Cortical activation of the SMC

No significant main effect of either group ($F = 0.70$, $P = 0.407$; $F = 0.11$, $P = 0.742$) or any of the conditions ($F = 1.52$, $P = 0.229$; $F = 0.57$, $P = 0.519$) were found for ΔHbO in the regions of interest (ROI) level SMC of the right ($_r$SMC) and left hemispheres, respectively, nor was there a significant interaction effect ($F = 0.22$, $P = 0.750$; $F = 1.18$, $P = 0.304$). As for the individual channels in SMC region, the main effect of condition on ΔHbO ($F = 4.90$, $P = 0.019$) in the $_r$SMC-CH34 did not pass the corrected alpha threshold ($P < 0.05/4$), nor was a significant group main

effect (F = 0.21, *P* = 0.651) or interaction effect (F = 0.40, *P* = 0.608) observed.

2.2. Cortical activation of the SMA

In the SMA over the right hemisphere ($_r$SMA), we observed a significant main effect from the experimental conditions on ΔHbO at the ROI level (F = 3.74, *P* = 0.042) and in the $_r$SMA-CH19 (F = 7.16, *P* = 0.004 < 0.05/2). Because no significant difference was found between groups in terms of their ΔHbO values, all participants were combined into one group, and a post-hoc analysis was conducted by performing pairwise comparisons between conditions, with Bonferroni adjustments (Fig. 2).

There were three movement-based conditions: left upper limb active movements with extrinsic motor performance visual feedback (LAV); left upper limb active movements with no extrinsic motor performance visual feedback (LAnV); and LP. Post-hoc analysis showed that, at the ROI level, ΔHbO during the LP condition was significantly lower than that during the LAV (mean difference [95% CI]: -0.158 [-0.167, -0.033]; *P* = 0.009) and LAnV conditions (mean difference [95% CI]: -0.146 [-0.282, -0.011]; *P* = 0.009). However, activation in this area was not significantly different between the LAV and LAnV conditions. Channel-level analysis revealed that in the $_r$SMA-CH19, significant differences were observed in the concentration changes of HbO between the LAV and LP conditions (mean difference [95% CI]: 0.208 [0.080, 0.335]; *P* = 0.001), as well as between the LAnV and LP conditions (mean difference [95% CI]: 0.182 [0.048, 0.316]; *P* = 0.005) (Fig. 2).

In the SMA region over the left hemisphere ($_l$SMA), neither a main effect nor an interaction effect was significant in the ROI-level analysis, but a significant main effect of condition was observed in the $_l$SMA-CH4 (F = 4.40, *P* = 0.016 < 0.05/2). However, no significant differences were

found in the pairwise comparisons after Bonferroni correction.

2.3. Cortical activation of the PMC

A two-way ANOVA revealed a significant main effect of the conditions on the ΔHbO values in the right PMC ($_r$PMC) (F = 3.98, P = 0.023), and the underlying channel, $_r$PMC-CH24 (F = 4.09, P = 0.027), did not pass the corrected alpha threshold ($P < 0.05/2$). Post hoc analysis demonstrated that the LAV condition resulted in significantly higher ΔHbO values in the $_r$PMC than in the LP condition, with a mean difference of (95% CI) 0.199 (0.072, 0.326; P = 0.001). Similarly, the ΔHbO value during the LAnV condition also increased significantly in the $_r$PMC compared with during the LP condition, with a mean difference of (95% CI) 0.162 (0.018, 0.307; P = 0.023). No significant changes in HbO concentration were found in the comparisons between the LAV and LAnV conditions in the $_r$PMC (Fig. 2).

3. Discussion

3.1. Summary of findings

Our experiment examined cortical activation in the SMC, SMA, and PMC during different upper-limb movement conditions in stroke patients and their healthy counterparts. Contrary to our first hypothesis, we found that, during different paretic upper limb movements, the levels of cortical activation in the SMC, SAM, and PMC of the bilateral hemispheres were not significantly different between stroke patients and healthy controls. Interestingly, previous studies have reported that movements of the affected upper extremity increased the likelihood of higher motor-related activation in the contralesional primary motor cortex, bilateral ventral PMC, and SMA relative to

such activation in healthy individuals (Favre et al., 2014; Kokotilo et al., 2009; Rehme et al., 2012). These studies have found that increased activity in sensorimotor areas is a common phenomenon in stroke patients and can be influenced by the severity of upper limb hemiparesis (Kokotilo et al., 2009; Rehme et al., 2012). Furthermore, sensorimotor area hyperactivation may return to normal levels in some well-recovered patients after chronic stroke (Buetefisch, 2015; Favre et al., 2014). In addition, most stroke patients have a high level of motor function recovery in their affected upper extremities, which was confirmed by the FTHUE in our experiment, with 80% of our stroke patients achieving levels 5 to 7 and 11 of them achieving level 7. At levels 5–7, stroke patients have mild upper-extremity impairment. They can perform isolated movements in their hemiplegic upper limbs to perform daily living tasks such as eating with a spoon, putting a box on the shelf, drinking from a glass, key turning, and manipulating chopsticks. Thus, the activation of cortical sensorimotor areas during upper limb movements in these patients might have returned to a normal level that was comparable to that in healthy controls.

Moreover, different methods of quantitative analyses may contribute to the inconsistencies in the between-group differences. A recent fNIRS study reported that the average amplitude parameter showed no significant group difference between stroke patients and healthy subjects, whereas the area under the curve parameter indicated greater cortical activation in stroke subjects than in healthy subjects (Lim and Eng, 2019). The parameter calculated in the present study for capturing possible differences in hemoglobin responses was the mean of the fNIRS signal during a task block. Taking average values during task blocks as data has been a frequently used approach in previous fNIRS studies for statistical analysis (Bai and Fong, 2020; Pelicioni et al., 2020; Yang et al., 2020). This method is recommended because it is less prone to being affected by artifacts (Vitório et al., 2017)

and is considered to be an appropriate method to use, with no assumptions of the exact shape or timing of the course of hemoglobin concentration changes (Tak and Ye, 2013). Thus, appropriate signal processing approaches and analyses of fNIRS data are necessary to guarantee accurate estimation of cortical activation.

Our second hypothesis, which stated that moving the upper limb actively would activate sensorimotor areas more than moving the limb passively, was supported by the results of the present study, where significantly higher ΔHbO in the PMC and SMA was observed during the LAV and LAnV conditions than in the LP condition. This indicates that active upper-limb movement, regardless of whether it was with or without extrinsic motor performance visual feedback, led to significantly greater cortical activation in the contralateral motor area when compared with passive upper-limb movement. Cortical activation in the contralateral PMC and SMA has also been observed in other fNIRS studies that focused on active (Yang et al., 2020; Yeo et al., 2013; Yokoyama et al., 2019) or passive upper limb motor tasks (Chang et al., 2017). Previous studies have reported that the cortical areas activated due to active and passive upper limb movements are similar (Szameitat et al., 2012), but active motor tasks led to significantly greater activation in motor areas than induced passive motion (Favre et al., 2014; Rehme et al., 2012). Our subjects were directed to perform upper limb millstone-turning movements on a robotic device, during which movements of the shoulder joint, elbow joint, and wrist joint of the hand were performed. The musculature of the hand, elbow, and shoulder joints is controlled by the primary motor cortex (PMC) in the human brain through the corticoreticulospinal and corticospinal tracts (Borot et al., 2018; Yeo et al., 2013). Thus, activation of these motor areas is observed during active or passive motion of the upper limb. However, active motion of the upper limb requires more muscle contraction than

passive motion, and thus, might yield higher cortical activation (Miyai et al., 2006). From the above findings, it appears that active upper limb motor training should be highly recommended for stroke patients in order to improve their upper limb motor function if they are medically stable, as well as to be able to meet the functional requirements of participating in active motor training.

Regarding the provision of extrinsic motor performance visual feedback in the LAV condition, additional activation was observed in our data in the contralateral PMC and SMA regions, in comparison to the findings in the regions with no provision of extrinsic visual feedback during the LAnV condition; however, the difference was not statistically significant. This is contrary to our third hypothesis, which indicates that extrinsic motor performance visual feedback does not lead to significant additional cortical activation in the sensorimotor areas. The extrinsic motor performance visual feedback provided in our experiment is known as "knowledge of performance" (Subramanian et al., 2009). The effect of knowledge of performance on the improvement of both motor performance and movement quality can last up to 1 month post-training. Moreover, providing visual feedback can lead to better retention of learned skills and enhanced motor learning (Subramanian et al., 2009; van Vliet, 2006). Some studies have reported that the PMC, SMA, and SMC are implicated in implicit motor learning (Grafton et al., 2002; Jang et al., 2005), and it is thought that PMC activation might enable patients to adjust their motor performance by using extrinsic feedback (Lin et al., 2013). Therefore, repeated practice may have accumulated the additional and mild PMC and SMA activation that was induced by providing extrinsic motor performance feedback in our study, and it may have established more durable connections to facilitate adaptive plasticity and ultimately improve upper limb motor function through motor learning in clinical rehabilitation.

In previous fNIRS studies, sample sizes ranged from 9 to 25, with one or two groups (Lim and

Eng, 2019; Yang et al., 2020; Yeo et al., 2013; Yokoyama et al., 2019). Our study included 40 healthy/stroke participants (20 participants per group). With this sample size, the post-hoc power analysis showed that the two-way ANOVAs of the $_{r}$SMA, $_{r}$SMA-CH19, $_{l}$SMA-CH4, and $_{r}$PMC regions had > 99% power (99.4%, 99.9%, 99.8%, and 99.6%, respectively), showing significant main effects within conditions with effect sizes of 0.32, 0.44, 0.34, and 0.33, respectively. This suggests that the sample size of the current study was acceptable.

3.2. Limitations

We acknowledge some limitations of the current experiment. First, because we used a convenience sampling method, more men than women were recruited into the stroke group. Since the differences in cerebral blood flow between men and women need to be considered (Aanerud et al., 2016), we statistically controlled for sex by setting it as a factor. However, in future research, the importance of homogeneity between groups should be noted prior to participant recruitment by choosing appropriate sampling methods. Second, compared with stroke patients, the healthy group with good upper limb motor function may have performed more repetitions when moving actively in the LAV and LAnV conditions. Moving the upper limb with more repetitions during the same time period induces greater cortical activation. To conduct further analyses that estimate how motor performance influences cortical activation during motor tasks, we recommend collecting the motor performance-related data of the participants during the three conditions—something that we were not able to consider in the current study. However, the current study made some efforts to control for the differences in repetition within patients and between the two groups. First, the target speed during the LAV and LAnV conditions was set at 20 rpm, which was a suitable speed for most stroke patients in our clinical rehabilitation. Second, we recruited stroke patients with mild to moderate

upper extremity impairment to ensure that their upper limb motor function could fulfill the three movement conditions. The results of our study also showed that most of the stroke patients in the current study had achieved good motor function recovery in their affected upper extremities, which may have reduced the difference between their motor performance and that of their healthy counterparts.

3.3. Conclusions

In this study, the level of cortical activation in stroke patients during active upper limb movement, with or without extrinsic motor performance visual feedback, and in stroke patients during passive upper limb movement was comparable to that in age-matched healthy controls. Meanwhile, active upper-limb movement appears to induce higher activation than passive movement in the contralateral PMC and SMA regions. Further research is required to determine the cumulative effect of extrinsic visual feedback on motor function recovery and neuroplasticity in stroke patients with varied upper limb motor functions.

4. Methods

4.1. Participants

Twenty patients (mean [SD] age = 59.9[12.0] years, 16 men) from the Shanghai YangZhi Rehabilitation Hospital, and 20 healthy subjects (mean [SD] age = 62.4[7.6] years, 8 men) comprised the study sample. All participants were right-handed, according to self-reports, and were able to follow the experimental instructions and complete all upper limb movements in the experiment. In addition, stroke patients met the criteria of being medically stable and without any

history of other neurological, orthopedic, or psychiatric illnesses. The experiment was conducted in accordance with the ethical principles of the Declaration of Helsinki (Christie 2000). All participants provided written informed consent prior to data collection. The study was approved by the Ethics Committee of YangZhi Rehabilitation Hospital (No.: YZ2002-016).

4.2. Experimental design and procedure

The experimental setup is illustrated in Fig. 3a. The participants sat comfortably in an adjustable chair in a quiet room. A computer was placed on a table approximately 1.2 m away from the participants. They completed a motor task on an upper-limb multi-joint movement robot (ZEPU-K2000K, Shandong ZEPU Medical Technology Co., China) during data collection. This training robot allows users to perform millstone-turning upper limb movements with their shoulder, elbow, and wrist joints when motion occurs in the limb. To standardize the conditions while conducting the upper limb motor task, the training robot was placed in front of and on a sagittal plane relative to the subject. The upper limb position relative to the handle and forearm pallet was organized such that elbow joint flexion achieved a maximum range of motion of 90° to 100° throughout the entire upper limb movement. While the upper limb was moving, no additional resistance was provided, and extrinsic motor performance visual feedback was provided continuously via a monitor on the robot, which showed the handle's actual speed of revolution.

Three movement-based conditions were used: LAV, LAnV, and LP. In our experiment, participants were instructed to place their left upper limbs (the paretic limbs in stroke patients) securely on the handle and forearm pallet of the training robot and perform a millstone-turning movement. In the LAV condition, participants were instructed to perform the movement actively

and adjust their turning speeds to match the target speed of 20 rpm by looking at the actual speed shown on the monitor. The setups of the LAnV condition were similar to those of the LAV condition, but the extrinsic motor performance visual feedback for the actual turning speed on the monitor was covered when the actual speed matched the same target speed. In the LP condition, participants were asked to relax their upper limbs and allow the handle and forearm pallet, which were driven by a motor, to move their limbs freely. In this case, the target speed of the motor was set to 20 rpm. The target speed during the LAV and LAnV conditions was set at 20 rpm because it was a suitable speed for most stroke patients in our clinical rehabilitation.

Before data collection, a practice session was conducted to allow participants to familiarize themselves with the three conditions and instructions. To avoid any bias from the order of the conditions, the order was randomized for each participant using E-Prime software (version 3; Psychology Software Tools, Inc.). A block design paradigm was applied for fNIRS data collection (Fig. 3b). Each condition was conducted after 50 s of rest, which was used as the baseline period to stabilize the fNIRS signal. In addition, between the administrations of each condition, participants could take a 2-minute break. Each condition included three blocks, and each block started with a 30-s motor task period followed by 35 s of rest. During all rest periods, participants were instructed to focus motionlessly on a red cross on a white background presented by a computer monitor. A "ding" tone made by the E-Prime software was used to signal the start and end of each block.

4.3. fNIRS data acquisition and analysis

Cortical activity was measured using a continuous-wave optical system (NirScan; Danyang Huichuang Medical Equipment Co., Ltd, Jiangsu, China). The system emitted light at 730- and 850-

nm wavelengths to measure ΔHbO and concentration changes of deoxyhemoglobin, and the sampling rate was set at 11 Hz. A total of 36 channels (CHs), made up of 13 sources and 13 detectors, were symmetrically positioned to allow measurement of the central and lateral cortices (Fig. 4).

To define the coordinates of all the acquired channels, a 3D digitizer was utilized for localization, and the spatial registration of the 36 channels on the Montreal Neurological Institute (MNI) standard brain was imported into the NIRS-SPM toolbox (available at: https://www.nitrc.org/projects/nirs_spm/) (Ye et al., 2009) in MATLAB (The MathWorks, USA) for calculation. Based on the estimated spatial registration on MNI, we selected six ROIs. For the SMA, CHs 4 and 12 in the left hemisphere and CHs 19 and 20 in the right hemisphere were selected; for the left PMC, CHs 8 and 11 were selected; for the right PMC, CHs 23 and 24 were used; and for the left SMC, CHs 9, 10, 13, and 14 were selected, while for the right SMC, CHs 28, 30, 31, and 34 were chosen.

In our study, HbO was used as a marker for hemodynamic changes in regional cortical activation, as previous studies have shown that HbO is more sensitive to cerebral regional blood flow signal changes than deoxyhemoglobin concentration changes (Hoshi et al., 2001; Strangman et al., 2002); additionally, HbO is known to be especially sensitive to locomotion-related changes (Suzuki et al., 2004; Suzuki et al., 2008). The fNIRS data were analyzed using the Homer2 open-source software package, and the following steps were performed: (1) raw intensity data were converted to optical density change data; (2) to detect and correct motion artifacts caused by movement of the head, eyebrows, or jaw during data collection, the spline interpolation algorithm was utilized; (3) in order to remove noise caused by physiological components and low-frequency signal drifts from each wavelength, the low-cutoff bandpass filtering was set at 0.008, and the high-cutoff filtering was set

at 0.1 Hz; finally, (4) the optical density change data were converted to ΔHbO for each channel, according to the modified Beer–Lambert law (Bai et al., 2020; Bai and Fong, 2020).

A period of 45 s (beginning 5 s before the onset time of a block, which was used as the baseline, and continuing for 40 s after the onset time of the block) was cut as a temporal window for averaging the three blocks of each condition. Because changes in the concentration of HbO take approximately 4 to 6 s to reach a maximum after they begin to increase from baseline, and as they have an undershoot period of 10 to 30 s, a 20-s time interval between 5 and 25 s after the block onset was selected for the calculation of the mean ΔHbO induced by the three experimental conditions (Bai et al., 2020; Buxton et al., 2004).

4.4. Statistical analysis

Prior to the parametric tests, normal Q-Q plots and box plots were used to screen for outliers. Data beyond three standard deviations from the mean were considered outliers (Minati et al., 2011a; Minati et al., 2011b). Moreover, whether deviant data points were retained or discarded in the following statistical analysis was determined after carefully checking the data-collection process, evaluating the influence of outliers by Cook's distance, and running all statistical analysis with and without outliers. The Cook's distances of deviant data points that are greater than 1 might be considered as highly influential outliers (Cook and Weisberg, 1982). In running statistical analysis without outliers, multiple imputation analysis was used to impute the missing values after removing the deviant data points (Blankers et al., 2010). In the multiple imputation analysis, demographic variables (age and sex) and ΔHbO variables of all channels during LP condition, from the healthy group, were used. Both histogram plots and Shapiro–Wilk tests were used to check the normality

of the variables. Chi-square and independent *t*-tests were used to compare the demographic data between the groups. The mean ΔHbO of each individual CH that served as an ROI and their average, as ROI-level regional changes, were analyzed to compare the degree of regional activation under each of the three conditions. To determine whether the mean changes in HbO in the ROIs were significant between the groups and among the three motor conditions, two-way repeated-measures ANOVA was performed. Channel-level ANOVAs were also performed to check their consistency with ROI-level ANOVAs. The average change in HbO in the ROIs was the primary outcome in the current study. All ANOVAs were performed with sex as a factor, and the significance level was set at $P < 0.05$ for the ROI-level analysis and as $P <$ corrected alpha threshold (0.05 / the number of channels for each ROI) for the CH-level analysis. Post hoc analysis was conducted using pairwise comparisons between conditions with Bonferroni corrections. All statistical analyses were performed using SPSS (version 20.0; SPSS Inc. IL, USA, Chicago). The BrainNet View toolbox in MATLAB (MathWorks, USA) was used to visualize the statistical results (Xia et al., 2013).

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Declaration of Competing Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure captions

Fig. 1. Concentration changes of oxyhemoglobin (ΔHbO) during three conditions. (a) Averaged ΔHbO of the stroke and health in conditions. (b) The line charts of baseline-corrected time course curves between -5 to 40 s relative to the onset of conditions of the stroke and health. SMC, sensorimotor cortex; SMA, supplementary motor area; PMC, premotor cortex; LAV, left upper limb active movements with extrinsic motor performance visual feedback; LAnV, left upper limb active movements with no extrinsic motor performance visual feedback; LP, passive left upper limb movement; ROI, region of interest; $_{r}$, right hemisphere.

Fig. 2. Post hoc analysis by performing pairwise comparisons between conditions in the bar charts (mean ± SD with scatter plots to show all data points), and baseline-corrected time course curves between -5 to 40 s relative to the onset of conditions of all participants in the line charts. *****: $p < 0.05$, with Bonferroni adjustment for multiple comparisons; ******: $p < 0.001$, with Bonferroni adjustment for multiple comparisons; SMA, supplementary motor area; PMC, premotor cortex; LAV, left upper limb active movements with extrinsic motor performance visual feedback; LAnV, left upper limb active movements with no extrinsic motor performance visual feedback; LP, passive left upper limb movement; ROI, region of interest; CH, channel; $_{r}$, right hemisphere; $_{l}$, left hemisphere.

Fig. 3. The experimental design. (a) Experimental setup; (b) experimental procedure. Participants randomly performed three left upper limb motion tasks, each condition was consisted by three

blocks. Each block began with a period of 50 s as baseline and a 30 s motion period followed by a 35 s rest period. In the passive motion condition, the target speed of the motion was set to 20 rpm and driven by the machine; while in two active motion conditions, participants turned the training robot to match the target speed.

Fig. 4. Arrangement of the fNIRS optodes and channels. (a) The optode design. (b) The location of channels.

Table captions

Table 1. Demographic and disease-related information of participants.[a]

CrediT author statement

Weili Xia: Conceptualization, Software, Investigation, Methodology, Formal analysis, Writing-Original Draft. **Rongxia Dai:** Conceptualization, Methodology, Formal analysis, Writing-Original Draft. **Xiaojin Xu:** Methodology, Investigation. **Baoyu Huai:** Methodology, Investigation. **Zhongfei Bai:** Conceptualization, Writing-Review & Editing. **Jiaqi Zhang:** Conceptualization, Writing-Review & Editing. **Minxia Jin:** Software. **Wenxin Niu:** Conceptualization, Supervision, Project administration, Writing-Review & Editing, Funding acquisition.

Table 1.

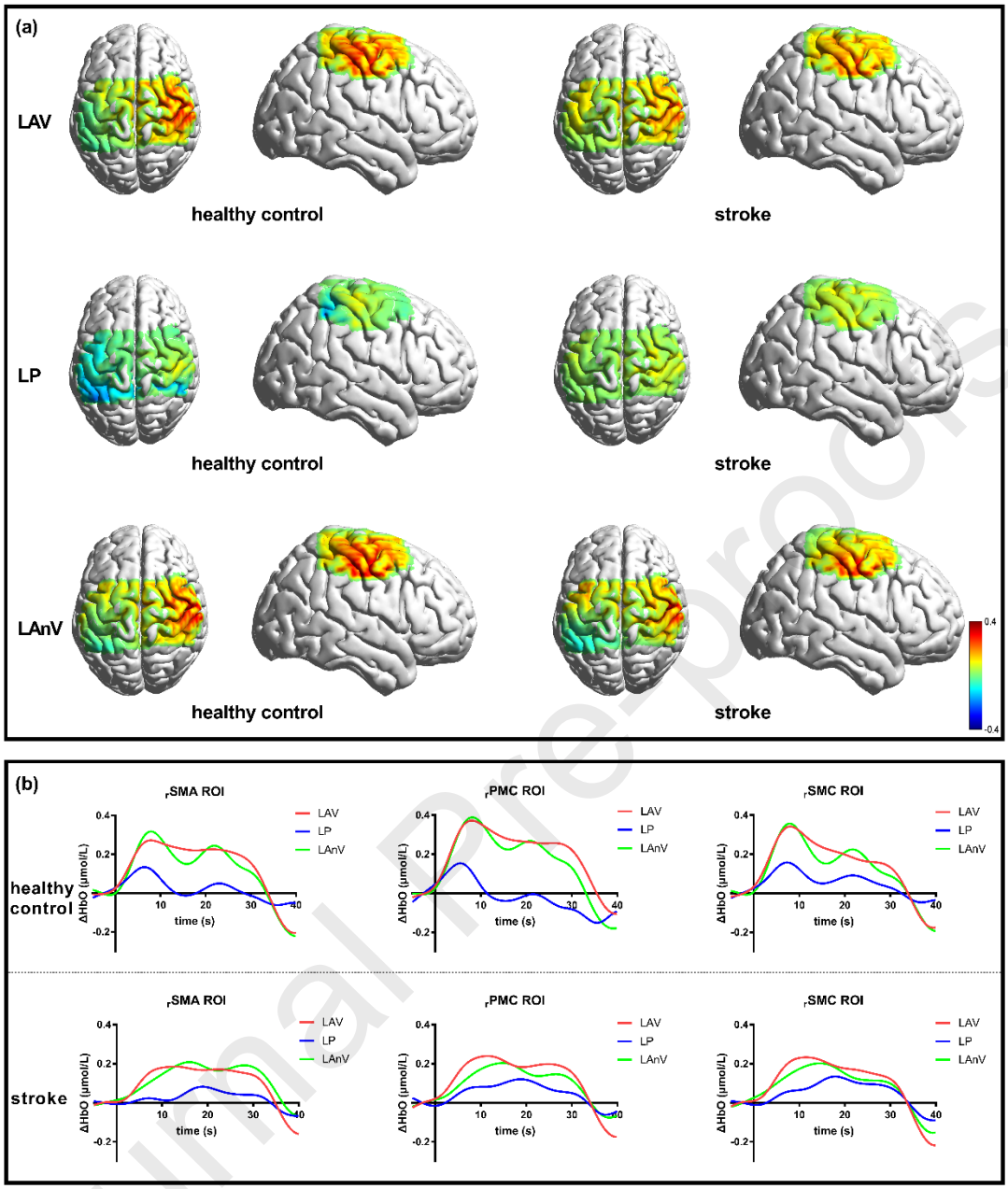
Demographic and disease-related information of participants.[a]

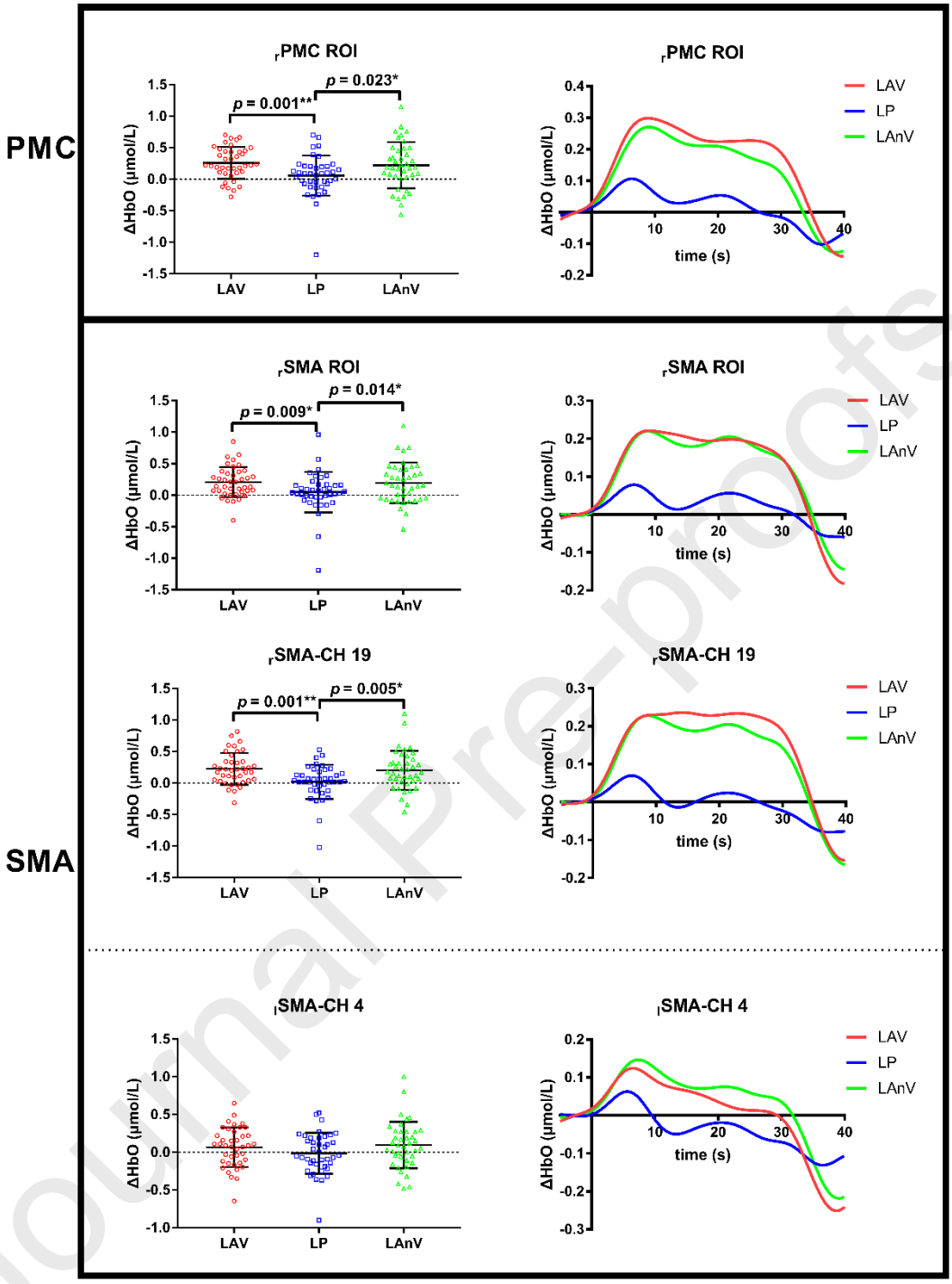
	Health (N=20)	Stroke (N=20)	*P*
Demographic			
Gender (%Male)	8 (40)	16 (80)	0.022*
Age	62.4 (7.6)	59.9 (12.0)	0.437
Clinics			
FTHUE (%)			
level 1	--	0 (0)	
level 2	--	0 (0)	
level 3	--	1 (5)	
level 4	--	3 (15)	
level 5	--	2 (10)	
level 6	--	3 (15)	
level 7	--	11 (55)	
Disease-related			
Disease duration (days)	--	222.7 (256.0)	
Diagnosis (%)			
cerebral hemorrhage	--	5 (25)	
cerebral infarction	--	15 (75)	

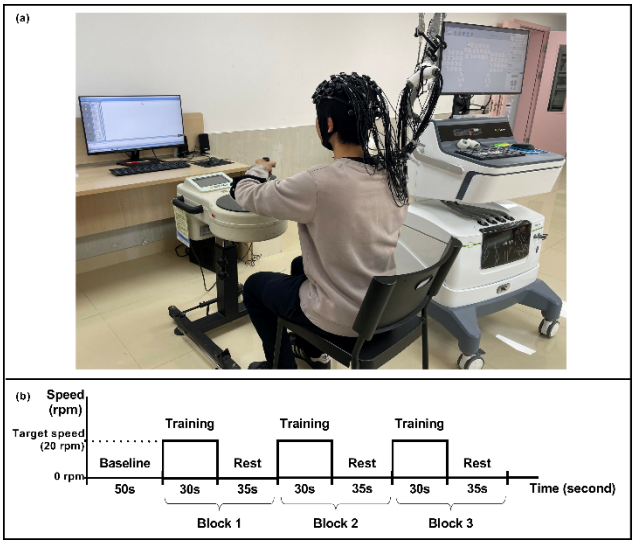
Abbreviations: FTHUE, the Functional Test for the Hemiplegic Upper Extremity.

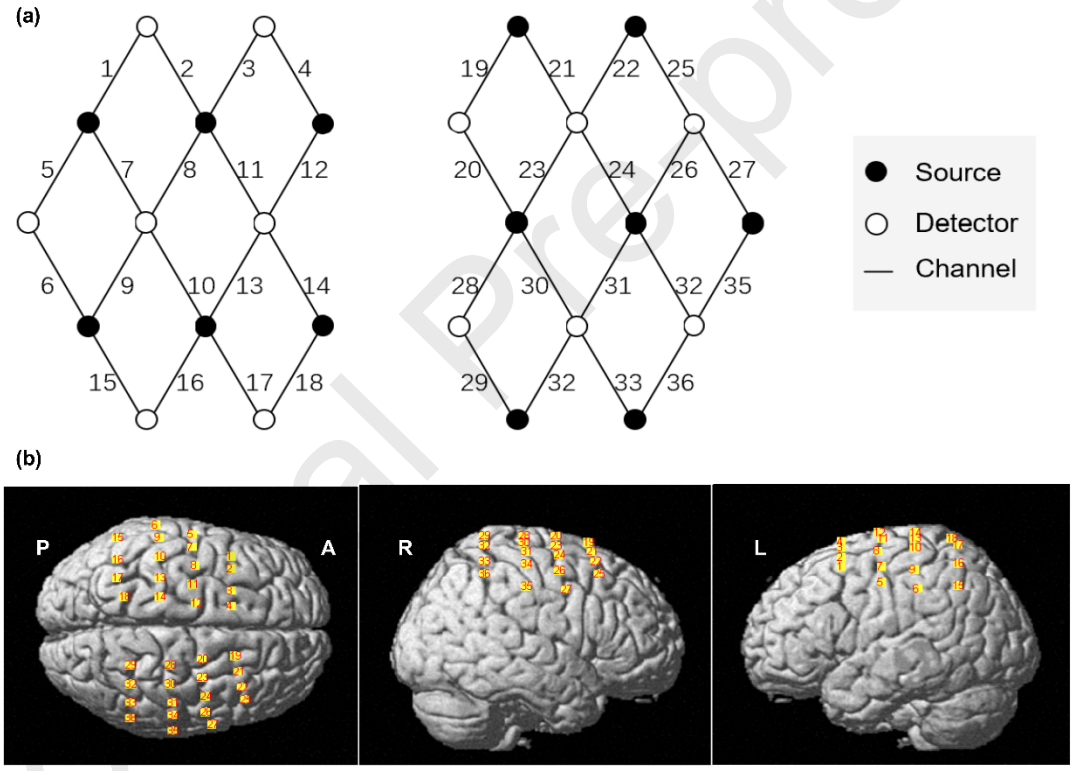
[a]Data are mean (SD) unless stated otherwise.

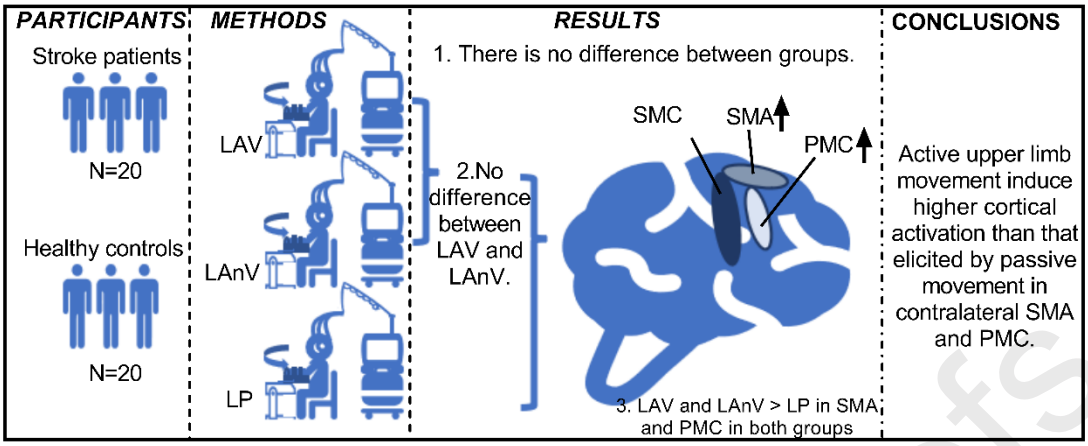
*$p < 0.05$.











HIGHLIGHTS

- Active upper limb moving induce higher cortical activation than passive movement.
- Cortical activation in the stroke patients was comparable to that in the healthy.
- Extrinsic visual feedback leads to additional activation in the contralateral PMC/SMA.