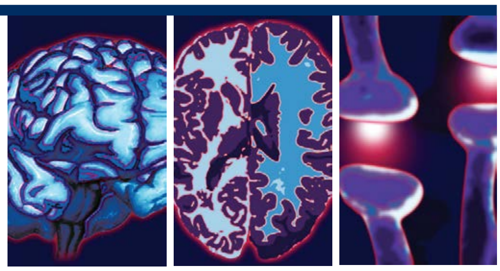
Research



Does Hand Posture Affect the Reliability and Reproducibility of Measures of Brain Function?

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ABSTRACT

Results from scientific studies of brain function have suffered from failure to replicate in subsequent studies. We hypothesize that, in order to obtain consistent results, it is necessary to standardize at least the posture and attention level in physiological studies of human brain activity associated with auditory perception. Here we focus on the effects of a minor postural changes on near-infrared spectroscopy (NIRS) measures of brain activity. In particular we recorded NIRS signals during "parallel" and "crossed" arm/hand positions. Six of 22 channels (4 on the left and 2 on the right hemisphere) showed significant level changes associated with hand crossing ($p<0.001$). Thus, even such simple postural changes may affect the reliability and reproducibility of the results of physiological study of brain function. We recommend that experimenters in physiological studies of speech perception require participants to keep their hands on a surface and feet on the floor.

Keywords

Brain Function, Hand posture, Auditory perception, Near-infrared spectroscopy, Neuronal activation

Introduction

Understanding the human brain and its functions remains one of the greatest scientific challenges. A major difficulty in studies of the brain, from the molecular to large-scale network level, is ensuring the reliability of results, since repeatability has been a problem in studies utilizing functional magnetic resonance imaging (f-MRI), near-infrared spectroscopy (NIRS), and positron-emission tomography (PET) [1]. Recent studies have indicated that establishing the reliability of key psychological findings would be a major contribution toward understanding the human side of the scientific process. For example, it has been reported that in only 39% of replication studies have the original results been unambiguously reobtained [2]. Researchers must undertake studies to identify factors that reduce reliability and conduct more carefully controlled studies to improve reliability. Funding agencies must also encourage such studies, and journals need to cover and publish them [3].

In a previous speech-and-hearing-related physiological study, we experienced problems with reproducibility [4]. Among the 70 volunteers in that study, we found that 14 (20%) of them fell asleep or changed their posture (hands and legs) during their sessions. Other reports have also indicated that volunteers' posture without attention could affect the outcomes of physiological studies [5,6]. Furthermore some reports suggested that it is necessary to standardize

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at least the posture and attention level in all studies of the human brain using physiological methods. In one physiological study, mind-wandering, and mindfulness are reported to differ even when the brain remains "silent" [7]. Another showed that crossing the hands over the midline impairs the ability to judge the order of a pair of tactile stimuli correctly when they are delivered in rapid succession to each hand. This impairment, termed the "crossed-hands deficit," has been attributed to a mismatch between the somatotopic and body-centered frames of reference, onto which somatosensory stimuli are automatically mapped [8]. Those reports suggested that it is necessary to standardize at least the posture and attention level in all studies of the human brain using physiological methods. To avoid this issue, we required volunteers in our previous study to maintain the same stable seated posture during the experiment.

In the present study, we examined the hypothesis that small postural changes could affect brain activity. We examined cerebral blood flow, which reflects neuronal activation, using NIRS in volunteers who were seated in front of a desk with their hands on the desk and feet firmly on the floor. At regular intervals, they were instructed to switch between normal (parallel arm) and crossed positions of the hands while otherwise maintaining a stable posture. If any effects of posture (two different hand postures) were observed, they could compromise the satisfactory replication of the results of brain studies.

Volunteers and Methods

■ Methods

This research protocol (R10-036R) ensured that all experiments were performed in accordance with relevant guidelines and regulations. It was approved by the National Hospital Organization, Tokyo Medical Center, Ethics Committee (the institutional review board of Tokyo Medical Center). Before participating in the study, all volunteers provided written informed consent.

■ Volunteers

We asked public institutions and universities, with no relationship with our institution to offer volunteers randomly. Inclusion criteria were good healthy condition, able to understand all instructions, and people who have not sinusitis or allergic rhinitis for volunteers. Initially, there were 30 Japanese volunteers for this study after those who had previously undergone facial or brain surgery were excluded. The group comprised 11 Japanese men (mean age 26.9 years and age range 23–32 years) and 19 Japanese women (mean age 28.4 years and age range 18–38 years). This group is consistent with random selection with respect to gender (p=.49), drawn randomly from the same age distribution (p=.97). Some of these were eliminated from the study (for reasons given below), so that the final group comprised 7 men and 7 women.

■ Equipment

Measurements were made with a 22-channel headband-type NIRS device (WOT-220 NIRS, Hitachi, Tokyo, Japan) that was developed to examine oxyhemoglobin levels of the brain, specifically the frontal lobe, via the forehead, since this area is free from obstruction by the hair. The 22 probe channels were attached to the volunteers' forehead just below the hairline, and the lower-central channel was aligned with Fpz of the International 10-20 system to record and compare activities based on blood oxyhemoglobin levels along the full lengths of the frontal lobes. The use of NIRS eliminates the acoustic background noise and discomfort associated with f-MRI and allows listening-based tests to be performed in a relatively natural (seated-posture) setting.

The equipment setup for this study is illustrated in **Figure 1**. The figure shows that the participant sat inside a soundproof chamber (AT-81, Rion, Tokyo, Japan). The experimenter was able to monitor the volunteers hands from outside the chamber through a window. Foot position was monitored with a video camera inside the chamber.

Two computers were used for event-related NIRS recordings. One was used to record NIRS signals with the measurement software of the Hitachi WOT-220 system. The second computer was used for stimulus presentation via Microsoft PowerPoint. A pulse at the start of each stimulus was used to synchronize the data recording with stimulus presentation. This was accomplished using a specially designed device simulating a mouse click in response to the synchronization pulse. Instructions to the volunteers ("parallel" or "crossed" hand position) were recorded at 44,100 samples/sec and presented diotically through EX880ST earphones (Sony Co. Ltd., Tokyo, Japan). The instructions were presented at the same comfortable level, measured as root mean square (rms) value.

Procedures

To satisfy circasemidian conditions, all volunteers were tested on a weekday afternoon after 14:00 at the National Hospital Organization, National Institute of Sensory Organs, Tokyo Medical Center. Participants sat inside the soundproof and the procedures were performed in a single session lasting approximately 7 min.

The time interval between successive instructions in each session was 30 sec., accurate to within 0.2 sec. The two instructions were presented alternately in the order "parallel" then "crossed," and this sequence was repeated 5 additional times. Data were collected for all 30 sec following each instruction, resulting in 360 sec per volunteer.

All volunteers were told to obey the following instructions: 1) sit at the desk and listen to the indication; 2) remain still, with your hands on the desk and feet on the floor with in a stable posture until you are instructed to change your hand position; and 3) avoid crossing your legs during the experiment. We compared the brain activity between the "parallel" and "crossed" hand positions.

To confirm that the instructions were followed, each session was monitored via the window from outside the chamber and via the video camera inside the chamber, as shown in **Figure 1**. Data from volunteers who were unable to follow the instructions or who fell asleep were excluded from the analysis. In total, 4 volunteers were excluded from the study for these reasons. Although the system had 10 channels on each side and 2 midline channels, for almost half of the remaining volunteer's sensors in some of the channels were not detected, and these participants were deleted from the study. For these reasons, a total 16 of the original 30 volunteers were excluded, leaving 14 volunteers (7 Japanese men and 7 Japanese women) who showed artifact-free data on channels except for the edge channels near the temporal regions. Channels that were not artifact-free were excluded, leaving 16 channels for analysis.

Analysis

The 30 seconds following a trigger point was considered a trial. For each trial, a 30 sec block of 22-channel data was collected. The data on the 16 artifact-free channels for analysis were calculated respectively by subtracting the mean signal during the initial 5 sec from the mean of the residual 25 sec signal. For each channel, the mean level change between the 25 sec during

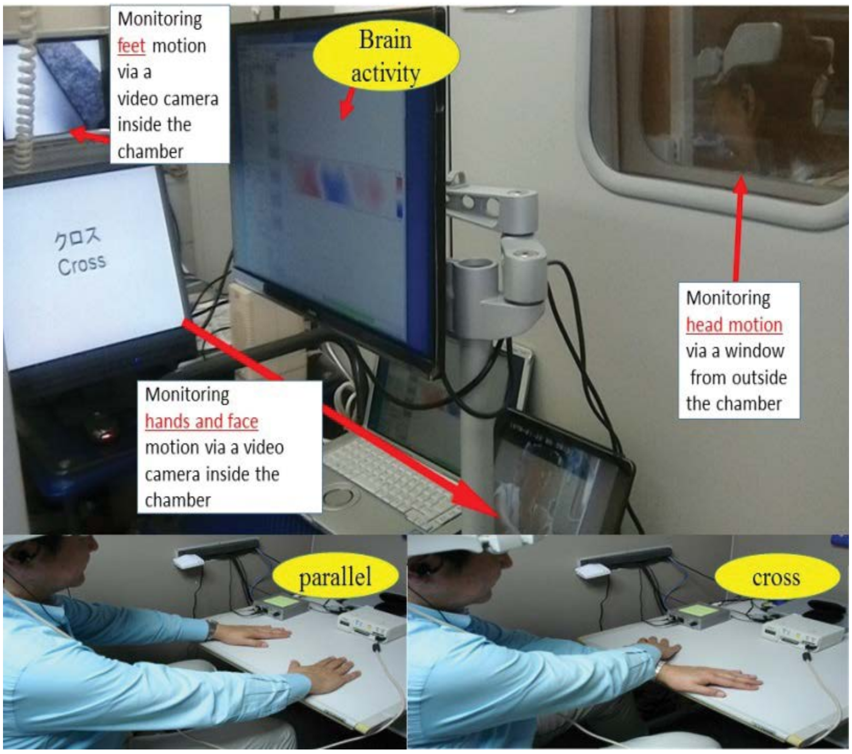

Figure 1: Each session was monitored via a window from outside the chamber and via a video camera inside the chamber.

crossed hand position from the 25 sec during the parallel position was adopted as the posture-related activation level.

We then performed a repeated measure ANOVA with two within-subject factors of the hand position (2 levels: parallel and crossed) and the sensor channel (16 levels) followed by post-hoc multiple comparisons with Bonferroni correction and matched-pair t-test on each channel between the two levels of hand positions to determine whether a statistically significant difference ($p < 0.05$) occurred. Subsequently, Pearson's correlation coefficient test was performed on the respective significantly different channels.

Result

The ANOVA results revealed that the hand position effect was statistically significant ($p < .001$) but the sensor channel effect and the interaction were not significant. The post-hoc comparisons failed to find significant between-channel differences. Analysis of the data from the 14 volunteers revealed that 6 channels (4 on the left hemisphere and 2 on the right) showed significant level changes associated with hand crossing **(Figure 2)**. There were mutual

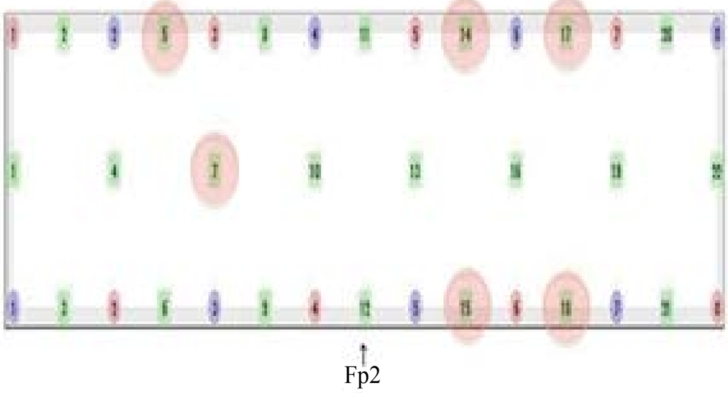

Figure 2: Significant channels marked with magenta circles. Left hemisphere: ch14 ($p < 0.05$), ch15 ($p < 0.05$), ch17 ($p < 0.05$), ch18 ($p<0.05$; Right hemisphere: ch5 ($p < 0.05$), ch7 ($p < 0.01$).

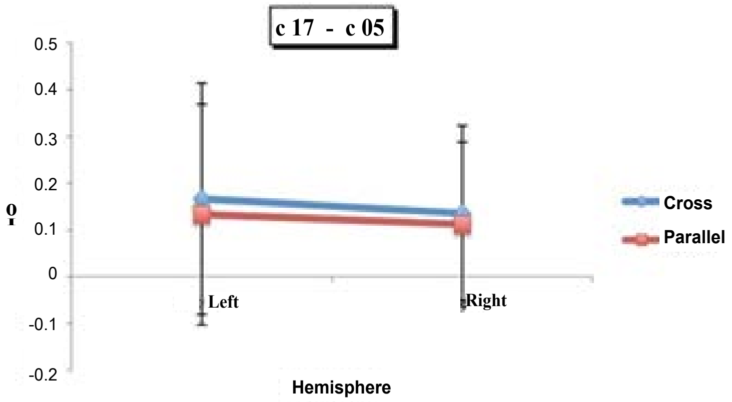

Figure 3: Activity levels on bilateral pair of significant channels.

Table 1: Correlation table of differences in hand position on respective significant channels (bold: $p < 0.05$; Ch.: channel).

	Left Front Polar				Right Front Polar	
	Ch 18	**Ch 17**	**Ch 15**	**Ch 14**	**Ch 7**	**Ch 5**
Ch 18	1.000	0.811	0.646	0.612	0.257	0.634
Ch 17	0.811	1.000	0.615	0.517	0.117	0.606
Ch 15	0.646	0.615	1.000	0.794	0.157	0.784
Ch 14	0.612	0.517	0.794	1.000	0.005	0.708
Ch 7	0.257	0.117	0.157	0.005	1.000	0.127
Ch 5	0.634	0.606	0.784	0.708	0.127	1.000

correlations among significant channels in the left but not in the right hemisphere. One of the significant right channels, channel 5, showed positive correlations with all significant left channels. However, the other significant right channel, channel 7, had no correlation with other significant channels **(Table 1, Figure 3)**.

Discussion

The purpose of this study was to clarify the possible importance of stable posture during experiments as it can affect the reliability and reproducibility of the results of physiological testing. Also in the clinical setting hand posture evaluated in the the possibility of novel approaches to the treatment of painful clinical conditions, crossing the arms over the midline was found to impair the multimodal processing of somatosensory stimuli and induce significant analgesia to noxious hand stimulation [9].

We experienced problems with reproducibility in speech-and hearing-related physiological studies, and previous results indicated that volunteers' posture could affect the outcomes of physiological studies [5,10]. To resolve that, we asked the volunteers in our studies (including this study) to maintain the same posture during speech and hearing trials [4,10]. During consecutive 30-sec trials, volunteers produced and maintained one of two stable sitting postures, differing only in position of the hands on a surface. Hand, foot, and head position were monitored to ensure that there were no other significant movements.

To prevent that volunteers fell asleep or changed their posture during their sessions [4], each session was monitored via a window from outside the chamber and via a video camera inside to ensure that the instructions were followed in the present study.

When people get worried, get confused, or try to relax, they sometimes fold their arms or cross their legs. Instead of those postures, we measured the differences in neuronal activities in the bilateral frontal lobes while the hands were placed in the "parallel" and "crossed" positions on a desk after receiving auditory stimuli in both the right and left ears instructing the volunteers to change hand positions. We were therefore able to observe the effects of hand crossing on the bilateral brain without the burden of performing intentional tasks. The scalp channels are estimated to reflect the cerebral frontopolar cortex (BA10) activity, [11,12] which was found to be closely involved in cognitive, communicative, and mental functions [10,13,14] The present results of hand crossing are consistent with those of previous studies suggesting frontopolar activation with arm crossing in f-MRI studies [6].

We found frontopolar asymmetry not only in terms of activity level but also in behavior. Two sets of right channels had prominent and no functional connections, respectively, with the corresponding 4 left channels. The front polar lateralization may cause more marked effects on brain activity with limb crossing [6]. Further research is required to confirm this mapping. Current EEG studies also showed the difference of brain activities between "parallel" and "crossed" hands positions [15,16].

Preventing postural changes, even just by crossing hands, is necessary to obtain reliable, reproducible results in all kind of physiological studies of the brain. To request volunteers to place their hands in a surface and feet on the floor and keep the same stable posture, even in physiological auditory perception studies, is important for increasing the reproducibility.

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Koichi Tsunoda wrote the main manuscript text, Sotaro Sekimoto and Mihiro Takazawa took charge of experiment with Koichi Tsunoda, and Kenji Itoh PhD mainly analyzed the experiment and prepared **Figures 2-3**. All authors reviewed the manuscript.

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