Transdural Doppler Ultrasonography Monitors Cerebral Blood Flow Changes in Relation to Motor Tasks

Monitoring changes in cerebral blood flow in association with neuronal activity has widely been used to evaluate various brain functions. However, current techniques do not directly measure blood flow changes in specified blood vessels. The present study identified arterioles within the cerebral cortex by echoencephalography and color Doppler imaging, and then measured blood flow velocity (BFV) changes in pulsed-wave Doppler mode. We applied this “transdural Doppler ultrasonography (TDD)” to examine BFV changes in the cortical motor-related areas of monkeys during the performance of unimanual (right or left) and bimanual key-press tasks. BFV in the primary motor cortex (MI) was increased in response to contralateral movement. In each of the unimanual and bimanual tasks, bimodal BFV increases related to both the instruction signal and the movement were observed in the supplementary motor area (SMA). Such BFV changes in the SMA were prominent during the early stage of task training and gradually decreased with improvements in task performance, leaving those in the MI unchanged. Moreover, BFV changes in the SMA depended on task difficulty. The present results indicate that TDD is useful for evaluating regional brain functions.

Keywords: bimanual movement, functional brain imaging, motor acquisition, primary motor cortex, supplementary motor area

Introduction

Since Roy and Sherrington (1890) first proposed the concept that brain blood supply is related to neuronal activity, a wide variety of modern imaging techniques for detecting regional changes in cerebral blood flow have been developed to assess activated brain regions during behavioral actions. For instance, positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) are widely used to explore diverse functions of the brain in humans and nonhuman primates (for PET, Deiber et al. 1991; Jenkins et al. 1994; Picard and Strick 1996; Weeks et al. 2001; Grafton et al. 2000; for fMRI, Petit et al. 1998; Deiber et al. 1999; Cunnington et al. 2002). These 2 leading techniques have successfully achieved functional brain imaging by monitoring activity-dependent metabolic changes in short-lived agents (H$_2$O, etc.) or by observing the blood oxygenation level-dependent (BOLD) effect, respectively (for reviews, see Paans et al. 2002; Logothetis 2003; Bagarinao et al. 2006; Otte and Halsband 2006).

The Doppler effect of laser or ultrasound waves is useful for measuring blood flow velocity (BFV) in the brain. In fact, the laser Doppler (LD) method (Williams et al. 1980; Iadecola and Reis 1990; Detre et al. 1998; Ances et al. 1999) and transcranial Doppler ultrasonography (TCD) (Deppe et al. 1997; Knecht et al. 2000) have been used to detect BFV changes in relation to neuronal activation. We have previously reported that ultrasonography is applicable to neuroanatomical and neurophysiological experiments. We obtained echencephalograms (computed ultrasound tomography of the brain) that showed brain structure and an injection needle or recording microelectrode based on reflectance of ultrasound. We also localized blood vessels by color Doppler imaging, depicting moving red blood cells in the specified region. Combined use of echencephalography and color Doppler imaging allows placement of an injection needle or recording microelectrode in a target site of monkey brain both accurately and safely, with no accidental injury to blood vessels (Tokuno et al. 2000, 2002; Tokuno and Chiken 2004; see also Glimcher et al. 2001).

In the course of these experiments, we have found that activity-dependent changes in BFV within visualized cerebral arterioles can be monitored by pulsed-wave Doppler mode. This method measures the real-time flow velocity of red blood cells to the acoustic transducer in small specified sites (for review, Griffith et al. 2004). The combination of these 3 methods, as “transdural Doppler ultrasonography (TDD),” could provide a potent methodological approach to real-time analysis of brain functions.

To test the validity of TDD, we attempted to measure BFV changes in the cerebral cortex of monkeys in relation to improvements in task performance and difficulty in motor tasks. In the present study, particular attention was paid to the supplementary motor area (SMA) on the medial wall of the frontal lobe. Accumulated electrophysiological and functional-imaging data indicate that the SMA plays a crucial role in bimanual coordination (Tanji et al. 1988; Matsuzaka et al. 1992; Kermadi et al. 1998; Ohara et al. 2000; Ullen et al. 2003; for review, see Tanji 1994, 2001). However, patterns of activity changes in the SMA during the improvement of task performance have remained poorly understood, probably due to the difficulty in achieving continual recordings from the same neuronal population.

To address this issue, we recorded BFV changes in the SMA during improvements in the performance of unimanual and bimanual motor tasks with discrimination, in comparison with those in the primary motor cortex (MI). After task performance reached a plateau level, we attempted to measure BFV changes in the MI and SMA that were related to task difficulty. The task was changed occasionally from complex discrimination to simple movement during BFV recordings on the same day.

Materials and Methods

Two female Japanese monkeys (Macaca fuscata) weighing 4.5 and 5.6 kg were used for the present study. All experiments were approved by the Animal Care and Use Committee of the Tokyo Metropolitan Institute for Neuroscience, Tokyo Metropolitan Organization for Medical Research, Fuchu, Tokyo 183-8526, Japan, the Division of System Neurophysiology, National Institute for Physiological Sciences, and Department of Physiological Sciences, The Graduate University for Advanced Studies, Okazaki, Aichi 444-8585, Japan, and CREST, Japan Science and Technology Agency, Kawaguchi, Saitama 352-0012, Japan.
Institute for Neuroscience and were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

**Task**

Each monkey was trained to perform a unimanual (right or left) or bimanual discrimination key-press task (discrimination task) according to visual instruction (Fig. 1). The discrimination task resembled that designed in a previous study (Tanji et al. 1988). Throughout all experiments, including task training and TDD recording, the monkeys sat in a primate chair in a dim room with shoulders and elbows restrained bilaterally by thermoplastic resin (Fig. 1B). Both hands were set on small keys, which the monkeys were allowed to press by minimal wrist and digit flexion. Three light-emitting diodes (LEDs; right, left, and bottom) were mounted on a panel in front of the monkeys and located close to one another (within 7.6° of visual field) to avoid unnecessary eye movements during task performance. Instruction signals were provided by illumination of the 2 upper LEDs. Illumination of either the right or left LED (2-s duration) meant unimanual key-press using one hand on the corresponding side, whereas illumination of both LEDs meant bimanual key-press using both hands (Fig. 1A). After a 1.5-s delay period, the lower LED representing the go signal was lit, requiring monkeys to perform key-press. In the bimanual key-press task, the time lag between right and left key-press onset was permitted within 180 ms. When monkeys performed the task correctly, the go signal was turned off and a small drop of fruit juice was then delivered as a reward. According to the hemisphere being recorded, types of key-press movements were classified as ipsilateral, contralateral or bilateral. These 3 types of movements were randomly presented to the monkeys.

The success rate (successful trials/total trials) of tasks reached 60% within 3 months. Daily recordings of BFV changes in the MI and SMA were then performed. BFV was monitored for a period of 2 months after reaching 60% success rate while the monkey’s performance improved. Success rate finally reached 95% after 5 months of training. When task performance reached this plateau level, tasks were occasionally changed from the discrimination task described above to a simple task during BFV recordings on the same day. Both monkeys performed 200 trials of the discrimination task (prechange period), and a return to the simple task was made. In the simple task, monkeys were required to repeat one of 3 movements (ipsilateral, contralateral, or bilateral) in response to light stimuli with no delay period for 300 trials. After 300 trials of the simple task, monkeys performed a further 200 trials of the discrimination task (postchange period).

Training and TDD recording sessions were performed for 3-5 h/day and 3 times per week. Body weight, food intake and other indicators of health were closely monitored throughout all experiments.

**Surgical Procedures**

After an initial training period, each monkey underwent a 2-step surgical operation for chronic TDD recording. Monkeys were anesthetized with ketamine hydrochloride (10 mg/kg, i.m.) and sodium pentobarbital (25 mg/kg, i.v.). Following depliation of the body including the head, face and forelimbs, monkeys were positioned in a stereotaxic apparatus. Under aseptic conditions, the skull was widely exposed and small stainless-steel screws were implanted into the skull as anchors. The exposed skull and screws were completely covered with transparent acrylic resin, and then 2 stainless-steel pipes were mounted parallel over the frontal and occipital lobes for head fixation. After recovery from surgery, task training was restarted.

Several weeks after first surgery, monkeys were sedated with a combination of ketamine hydrochloride (10 mg/kg, i.m.) and xylazine hydrochloride (1-2 mg/kg, i.m.) and seated in a primate chair with the head fixed in a stereotaxic frame attached to the chair. A large portion of the skull (5 x 6 cm) was then removed under aseptic conditions and a rectangular recording chamber (external dimensions, 7 x 8 cm) was fixed onto the skull with resin to cover the exposed brain area (Fig. 1C). To avoid postoperative infection and brain edema, monkeys received intravenous administration of antibiotics and mannitol during the operation.

**TDD Recording**

During each experimental session of the TDD recording, monkeys sat in the primate chair with the head fixed to the stereotaxic frame. An ultrasonographic device (LOGIQ 700MR; GE Medical Systems, WI) was used in the present study. Echoencephalography, color Doppler imaging and BFV data using the pulsed-wave Doppler mode were recorded through an acoustic transducer (M12L; bandwidth, 4.5-13.0 MHz; field of view, 39 mm; GE Medical Systems). In the present study, echoencephalography, color Doppler imaging and BFV data by the pulsed-wave Doppler mode were obtained using 13-, 6.8-, and 6.2-MHz ultrasounds, respectively. The acoustic transducer was held by a stereotaxic manipulator and positioned in a saline-filled recording chamber (Fig. 1C).

Echoencephalography displays various brain structures, including the dura mater, sulci, ventricles and white matter including the corpus callosum, based on the reflectance of ultrasound. The ultrasound wave is released from the acoustic transducer, and part of the wave is reflected from brain structures to the transducer. Time lag between the release of ultrasound and detection of the reflected wave indicates the depth of structures, whereas the magnitude of reflected waves indicates the reflectance of structures. Color Doppler imaging displays the location of vessels, including arterioles and veins, in a specified region. The frequency of reflected waves is shifted by the Doppler effect based on the moving velocity of red blood cells. Blood vessels were thus depicted as the velocity of red blood cells approaching or receding from the transducer (Yoshida et al. 1961; for review, Griffith et al. 2001). In color Doppler imaging, BFV data were averaged for 80-200 ms to obtain clear images. Width of averaged time was dependent on the size and depth of each region of interest. The pulsed-wave Doppler mode measures real-time BFV data (1.5-2 kHz of the sampling rate in the present study) within a small sampling volume (300 μm x 300 μm). The principle of the method is the same as that for color Doppler imaging, but the pulsed-wave Doppler mode uses cycles of ultrasound separated by gaps of silence, and provides information on
real-time BFV changes without averaging. According to comparisons between ultrasound images and histological measurements of vessels under light microscopy, we have estimated that the maximal spatial resolution in echoencephalography and color Doppler imaging is 100 μm, whereas that in pulsed-wave Doppler mode is 300 μm (see the technical considerations in the Discussion).

In our experiments, TDD recordings were performed using a combination of echoencephalography, color Doppler imaging and pulsed-wave Doppler mode. First, arterioles in the cerebral cortex (intracortical arterioles) that branched from pial arteries were identified using a combination of echoencephalography and color Doppler imaging. Intracortical arterioles could be distinguished from arteries and veins based on the following features: 1) intracortical arterioles are vertically located in the superficial layer of the cerebral cortex, and show a small diameter; and 2) BFV is higher in arterioles than that in veins and is synchronized with the heartbeat. Real-time, direct monitoring of BFV in intracortical arterioles was then performed by the pulsed-wave Doppler mode. The sampling gate is able to arbitrarily set the position and size of a sampling volume and was fixed at a minimum size (300 μm × 300 μm) through the experimental period. Doppler angle, as the direction of ultrasound releasing-receiving, was adjusted (between -60° and 60°) to obtain the maximum amplitude between systolic and intersystolic BFV. A Wall filter (cut-off frequency, 10–49 Hz) was adjusted under the level of intersystolic BFV to eliminate slow-frequency Doppler noise. The Wall filter does not affect measurement of BFV changes because: 1) the frequency of Doppler signal waves from arterioles is higher (>100 Hz) in the present condition; and 2) systolic BFV was used in the following analysis (see Data Analysis).

After task performance reached 95% of the success rate, TDD recordings that cover wide areas of the cerebral cortex were performed in one monkey (Monkey 1) to prepare functional unfolded maps during the discrimination task. For this purpose, the frontal and parietal cortices were divided into 1 mm × 1 mm bins based on echoencephalography. The location of each bin was determined by echoencephalography, color Doppler imaging and stereotaxic coordinates and fixed through all experimental sessions. BFV changes in cortical arterioles within each bin were then measured using the pulse-wave Doppler mode.

In addition electroneumography (EMG) was recorded through surface electrodes (total gain, ×2000; low-cut filter, 50 Hz; high-cut filter, 1.5 kHz; rectified) from the following muscles bilaterally: extensor and flexor muscles of the digits, triceps, biceps, deltoid, and paravertebral muscles. Field potential of the cerebral cortex was also recorded through a silver ball electrode on the dura mater and a stainless-steel screw implanted in the parietal bone as a reference electrode (total gain, ×20 000; low-cut filter, 0.08 Hz; high-cut filter, 300 Hz).

Data Analysis

Doppler signals obtained from the ultrasonographic device were stored in a computer for data analysis. Signals were converted to BFV data as follows (Fig. 2). First, the pulsatile BFV wave was obtained from Doppler signals using Fourier transform with the aid of Joint-Time-Frequency-Analysis software (Labview Toolkit; National Instruments, TX). The parameters of Fourier transform were adjusted to obtain maximum pulse amplitude in each recording site. Second, the systolic BFV wave was obtained by tracing pulsatile BFV data. Systolic BFV wave was obtained from the Doppler signal using Fourier transform. Finally, the 50-trial average of systolic BFV waves is represented as BFV changes, and mean ± SD of BFV changes during the control period (0.5-s duration, before the instruction signal) is calculated.

Figure 2. Schematic diagram showing the procedures for data analysis. First, the pulsatile BFV wave is obtained from the Doppler signal using Fourier transform. Second, the systolic BFV wave is obtained from pulsatile BFV data by tracing peaks. Finally, the 50-trial average of systolic BFV waves is represented as BFV changes, and mean ± SD of BFV changes during the control period (0.5-s duration, before the instruction signal) is calculated.

Results

Echoencephalography and Color Doppler Imaging

A typical echoencephalogram and color Doppler image of the monkey brain are shown in Figure 3. The banks of the cingulate sulcus were clearly observed on echoencephalography (Fig. 3A). In addition to large arteries and veins, intracortical arterioles were seen in the superficial layer of the cortex as color tips or strips depending on the orientation of blood vessels in color Doppler imaging (Fig. 3B). Resolutions of echoencephalograms and color Doppler images were dependent on depth from the acoustic transducer. In the frontal lobe,
for example, subcortical structures such as the thalamus and basal ganglia were not clearly identifiable (Figs 4A–C, 6A–C). In addition, acoustic reflection from the walls or base of the skull often interfered with images.

BFV Changes in MI and SMA

Forelimb regions of the MI and SMA were determined as areas that displayed BFV changes in response to wrist passive movements in the precentral gyrus and medial-wall cortex, respectively, using the pulsed-wave Doppler mode. Figures 4–6 show averaged and normalized BFV changes in the MI and SMA while Monkey 1 was performing the discrimination task well (success rate, 87–89%). In Figure 4, the sampling gate of the pulsed-wave Doppler mode was placed on an intracortical arteriole in the anterior bank of the central sulcus, corresponding to the forelimb region of the MI (Fig. 4A–C). Significant increases of 30–40% in BFV were detected in response to bilateral and contralateral movements (Fig. 4D). No BFV changes were found during the ipsilateral-movement task. BFV changes during the instruction and delay periods were sufficiently as to not exceed the 2 SD level of the control (Fig. 4D). BFV changes, EMG of the extensor and flexor muscles of the digits and field potentials in the MI during the contralateral movement task were recorded simultaneously (Fig. 5). BFV increases in relation to movement were observed after EMG and field potentials were changed (Fig. 5). Peak-to-peak time lags between BFV changes and EMG/field potentials were approximately 1.5 s (EMG of right-hand flexors, 1.46 s; field potential, 1.52 s). The duration of field-potential change was about 0.9 s, whereas that of BFV change was about 3 s (Fig. 5).

Figure 6 shows results for a sampling gate placed in the medial wall of the hemisphere, corresponding to the forelimb region of the SMA (Fig. 6A–C). For each of the 3 types of hand movements (bilateral, contralateral and ipsilateral), bimodal increases in BFV were detected in response to both instruction signals and movements (Fig. 6D). The first BFV increment occurred approximately 1.5 s after onset of the instruction signal, whereas the second increment occurred almost 1 s after movement onset (key-press). The largest BFV changes were observed in relation to the ipsilateral-movement task.

Figure 3. Coronal views of echocencephalography and color Doppler imaging. (A) A coronal plane echoencephalogram 25 mm anterior from the interaural line. (B) Color Doppler image from the same plane. Arterioles from the subarachnoid space to the cerebral cortex are easily observed. The square zone in (A) corresponds to that in (B). Red areas represent areas containing moving red blood cells approaching the acoustic transducer, whereas blue areas represent areas containing those moving away from the transducer. The scale for BFV is depicted in the lower left. Scale bar = 10 mm.

Figure 4. Typical BFV changes in the MI in response to bilateral and unimanual (contralateral and ipsilateral) movements. (A) Nissl-stained coronal section corresponding to (B) and (C) in the same monkey. CgS, cingulate sulcus; CS, central sulcus; Put, putamen; SPS, superior precentral sulcus; Th, thalamus. The rectangular zone in (A) corresponds to (B). (B) An echocencephalogram 15 mm anterior to the interaural line. (C) Color Doppler image of (B). The scale of BFV is depicted on the left. The white square indicates a sampling gate (300 μm × 300 μm) position for pulsed-wave Doppler mode in the anterior bank of the central sulcus corresponding to the distal forelimb region of MI. (D) Movement-related BFV changes in the MI during task performance. BFV change was averaged for 50 success trials. BFV data were aligned to onset of the instruction signal (time = 0). Significant increases in BFV were observed in bilateral and contralateral movements, but not in ipsilateral movement. Movement onsets are indicated as histograms.
BFV Changes during Improvement of Task Performance

To investigate how BFV changes described in the previous section had been altered, BFV changes in the MI and SMA during the improvement of task performance were examined (Fig. 7). BFV changes were repeatedly measured on the same intracortical arterioles in the MI and SMA from 3 to 5 months. Each arteriole could be identified with the aid of color Doppler imaging and BFV index [(systolic velocity-intersystolic velocity)/systolic velocity], which differed among arterioles in pulsed-wave Doppler mode. BFV changes were normalized to the BFV change at first recording.

In Monkey 1, the success rate gradually increased and reached a plateau at about 95% within 5 months (Fig. 7A). With respect to contralateral movements, the amplitude of movement-related BFV changes in the MI remained unaltered during improvements in task performance (Fig. 7B). Conversely, amplitudes of BFV changes in relation to the instruction signal and movement in the SMA gradually decreased (Fig. 7C) and finally reached 50% of the level at the early stage of training. Similar results were obtained in Monkey 2, although the amplitude of BFV decrement in the SMA was smaller (Fig. 8). In the other types of movements, BFV changes in the SMA in relation to the instruction signal and movement also decreased during improvements in task performance, whereas BFV changes of the MI during the bilateral movement task remained unchanged (data not shown).

BFV Changes in Error Trials

Error trials were divided into 4 groups depending on the keys pressed, that is, contralateral, ipsilateral and bimanual irregular key-press and no key-press. BFV changes in contralateral irregular key-press trials during the improvement of task performance are shown in Figure 8. BFV changes in the MI were observed with contralateral and bimanual irregular key-press, but not with ipsilateral irregular key-press (data not shown). BFV changes in the MI in response to contralateral and bimanual irregular key-press remained unchanged during improvements in task performance (Fig. 8). BFV changes in the SMA in relation to the instruction signal and movement were observed in all 4 error groups (data not shown). BFV changes in success trials in relation to movement did not

Figure 5. Temporal relationship among BFV changes, field potentials, EMGs, and actual movement in the contralateral movement task is shown. Data for 50 success trials were averaged. Peak-to-peak time lag between EMG of the right-hand flexors and BVF changes was 1.46 s, whereas that between field potential and BVF changes was 1.52 s.

Figure 6. Typical BFV changes in the SMA in response to bilateral and unimanual (contralateral and ipsilateral) movements. (A) Nissl-stained coronal section corresponding to (B) and (C) in the same monkey. Cd, caudate nucleus; spur, spur of the arcuate sulcus. The rectangular zone in (A) corresponds to (B). (B) An echoencephalogram 22 mm anterior to the interaural line. (C) Color Doppler image of (B). The scale of the BFV is depicted on the left. The white square indicates a sampling gate (300 μm × 300 μm) position of pulsed-wave Doppler mode in the medial wall corresponding to the forelimb region of SMA. (D) Task-related bimodal BFV changes in the SMA. BFV changes in relation to instruction signals and movements are seen in all types of movements.

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differ from those in error trials in all error groups (see Fig. 8 for BFV change in contralateral irregular key-press trials). BFV changes in relation to the instruction signal in contralateral and ipsilateral irregular key-press and no key-press error trials were significantly smaller than in success trials (see Fig. 8 for contralateral irregular key-press trials). BFV changes in the SMA in relation to the instruction signal and movement decreased significantly with improvements in task performance (Figs 7, 8).

**Mapping of BFV Changes**
Contralateral instruction signal- and movement-related BFV changes in discrimination task were charted on the unfolded map of the cortex (Fig. 11). In the frontal lobe, large increases in BFV related to the instruction signal were observed not only in the SMA, but also in the rostrally situated motor-related areas, such as the presupplementary motor area (pre-SMA), rostral cingulate motor area (CMAr) and rostral part of the dorsal premotor cortex (PMdr) (Fig. 11A), with the pre-SMA and SMA displaying the most prominent changes. Significant BFV increases were also seen in the cingulate gyrus, dorsal and ventral cingulate motor areas (CMAd, CMAv), caudal part of the ventral premotor cortex (PMvc) and dorsolateral part of the prefrontal cortex. No BFV changes in response to the instruction signal were found in the MI (see also Figs 4D and 5).

As described above, BFV increases in response to movement were clearly observed in the MI and SMA (Fig. 11B; see also Figs 4D, 5, and 6D). Significant changes in BFV were also

Figure 7. BFV changes in the MI and SMA during the improvement of task performance. (A) Success rate (success trials/total trials) of the discrimination task in Monkey 1. Each symbol represents success rate on a single day. Success rate reached 60-70% in the 3rd month and reached a plateau (about 95%) in the fifth month after the start of task training. Success rate is fitted to the exponential curve. In (B) and (C), BFV changes at a sampling gate (size, 300 μm × 300 μm) in the MI or SMA were averaged for 50 success trials, respectively. Location of the sampling gate was determined by echoencephalography, color Doppler imaging and stereotaxic coordinates and fixed through all experimental sessions. BFV changes in the MI and SMA were normalized to the baseline, integrated during the specified time windows (movement and instruction signal) and presented as percentages and SD (bars) in comparison with the earliest recording session (third month). *P < 0.001, one-way ANOVA.

Figure 8. BFV changes in success and error trials during the improvement of task performance in the MI and SMA. BFV changes were recorded from the same site and averaged for 50 success trials of contralateral movement and for 20 contralateral irregular key-press trials. BFV changes are presented as percentages compared with success trials in the earliest recording. *P < 0.001, one-way ANOVA.

**BFV Changes in MI and SMA in Relation to Task Changes**
Effects on BFV changes in the MI and SMA when tasks were switched from the discrimination task to the simple task were investigated (Figs 9, 10). Reaction time decreased when the task was changed from discrimination to simple and recovered after the task was returned to discrimination (Figs 9B, 10B). Movement-related BFV changes in MI during contralateral movements tended to decrease along with task change, but these decreases were not significant (Fig. 9C, D; see also Fig. 9A, 121–140 trials). Conversely, both movement- and instruction signal-related BFV changes in the SMA were significantly decreased (one-way ANOVA, P < 0.001) along with shortening of reaction time (Fig. 10C–E; see also Fig. 10A, 121–140 trials). When the task was returned to discrimination, BFV changes recovered (Figs 9C, D and 10C–E; see also Figs 9A post and 10A post).
seen in many other motor-related areas, such as the pre-SMA, CMAr, CMAd, and rostral part of the ventral premotor cortex. In addition, considerable BFV increases were observed in the primary and secondary somatosensory cortices (SI, SII). Similar patterns of activity distribution were obtained in both Monkey 1 (as presented here) and Monkey 2 (data not shown).

Discussion

In the present study, regional BFV changes in relation to unimanual and bimanual key-press tasks were monitored by TDD recording. Herein, we provide 2 major findings. First, patterns of BFV changes in motor-related areas examined in the present study resembled those of task-related neuronal activities examined previously by electrophysiological methods (Tanji et al. 1988; Aizawa et al. 1991; Mushiake et al. 1991; Matsuzaka et al. 1992; Halsband et al. 1994; Kermadi et al. 1998; Hoshi and Tanji 2004; for reviews, see Tanji 1994, 2001). This suggests that recorded ultrasound Doppler data may represent actual activity of a neuronal population in the recoding site, and TDD can thus be useful for functional brain imaging. Second, BFV changes in the SMA were decreased during the improvement of task performance. Decrements in BFV alterations in the SMA were also observed when the task changed from complex discrimination to simple movement. This suggests that the SMA plays important roles in higher-order cognitive functions, such as motor acquisition and motor selection, particularly in terms of bimanual behavior.

Technical Considerations

Pulsed-wave Doppler mode could detect BFV changes in an area of 300 μm × 300 μm based on estimation by ultrasonography and histological examination of blood vessels, and mainly identified BFV changes in intracortical arterioles. Arterioles in the cerebral cortex issue numerous branches over all layers of the cortex and branched arterioles (precapillary arterioles) are connected to the capillary networks that form anastomoses (Anderson and Anderson 1978; Bär 1980; Pawlik et al. 1981; Mchedlishvili and Kuridze 1984; Borowsky and Collins 1989; Edvinsson and Mackenzie 2002). In monkeys, a single arteriole is considered to supply blood flow to a cortical vascular column, which has a diameter of 500–1000 μm. The pulsed-wave Doppler mode thus has sufficient spatial resolution to detect BFV changes in a single cortical vascular column, and spatial resolution of the TDD is determined by the size of the cortical vascular column. The sampling rate of the pulsed-wave Doppler mode was 1.5–2 kHz. Temporal resolution of TDD depends on heart rate, as BFV changes in one trial are defined by tracing the peaks of pulsatile BFV data (Fig. 2). In the present study, heart rates of monkeys were 120–160 beats/min. The temporal resolution of TDD was estimated as 2–2.7 Hz.
Pulsed-wave Doppler mode thus also has enough temporal resolution to detect task-related BFV changes. When a group of neurons fires, deoxyhemoglobin is initially increased and then decreased if sufficient blood flow is present. The BOLD effect of fMRI detects differences in magnetic susceptibility between oxy- and deoxyhemoglobin. Many fMRI studies have measured decreased deoxyhemoglobin (positive BOLD signal; see Ogawa et al. 1990; Kwong et al. 1992; Otte and Halsband 2006), whereas the initial increase in deoxyhemoglobin (negative BOLD signal) can be measured using a high-magnetic field fMRI system (for reviews, see Logothetis 2003; Bagarinao et al. 2006). On the other hand, PET studies using short-lived radiopharmaceuticals, such as H$_2^{15}$O or C$_2^{15}$O$_2$, have measured blood flow changes after the initial increase in deoxyhemoglobin (for review, see Paans et al. 2002). TDD and these 2 prevailing functional brain-mapping techniques thus both observe the phenomenon of blood flow increases after hypoxoxygenation of neuronal and glial cells caused by brain activation. It may be concluded that TDD has several advantages over other existing imaging techniques, such as fMRI and PET: 1) this technique enables us to measure blood flow increase directly and identify anatomical structures simultaneously; 2) its spatial and temporal resolutions are almost relevant to anatomical and physiological limits, and

Figure 10. Effects of task change from the discrimination task to the simple task on BFV changes in the SMA. In this case, the task was changed to the simple contralateral movement task. (A) BFV changes during the performance of the discrimination task (upper), during the performance of the simple task (middle) and after a return to the discrimination task (lower) in Monkey 1. BFV changes were aligned at go signal onset and averaged for 20 trials. (B) Reaction time changes in Monkey 1 during task changes on the same day. (C) Movement-related BFV changes. (D) Instruction signal-related BFV changes in the SMA during task changes in (B). BFV changes were recorded continuously at the same sampling rate. Reaction time and BFV changes were averaged for 20 trials in (B) to (D). BFV changes were normalized to the baseline, integrated during the specified time windows (movement and instruction signal) and presented as percentages and SD (bars) in comparison with the prechange period. *$P < 0.001$, one-way ANOVA. (E) Comparisons of BFV changes between Monkeys 1 and 2. Data were averaged for 3 sessions of task changes.
event-related BFV changes can easily be recorded; 3) both cost and space can be saved. Conversely, TDD has 2 major disadvantages: 1) this method requires invasive treatment, that is, craniotomy; 2) it still has a low spatial resolution for deeply-situated brain structures.

Together with TDD, LD recording and TCD measure velocity changes in red blood cell flow (Detre et al. 1998; Ances et al. 1999). The ultrasound or laser is released from an acoustic transducer or laser probe, respectively, and part of the wave reflects from moving red blood cells. Based on the velocity of red blood cell movement, frequency of the reflecting wave shifts according to the Doppler effect (Yoshida et al. 1961). However, BFV changes in cortical arterioles are impossible to directly monitor using LD. As absorption is more intense for laser than for ultrasound, clear tomographic images indicating the precise location of intracortical arterioles cannot be obtained by LD. On the other hand, BFV changes in the middle cerebral artery can be measured noninvasively by TCD that uses 2-MHz ultrasound to penetrate into the cranium (Deppe et al. 1997; Knecht et al. 2000). Although the spatial resolution of TCD is not as high as that of TDD, this technique may easily be applied to gross analysis of the functional difference between hemispheres (see Knecht et al. 2000).

LD recordings from the rat somatosensory cortex have shown BFV changes approximately 1.5 s after electrical stimulation of the forepaw (Detre et al. 1998). This is in accordance with data obtained using a microscopic high-speed video camera system, showing that the diameter of pial arterioles in the hindlimb region of the rat somatosensory cortex is increased 1.4 ± 0.2 s after sciatic nerve stimulation (Ngai et al. 1988). In the present study, BFV changes were detected approximately 1.5 s after movement onset. The time lag observed in the present study agrees well with that reported by different methods. Recent studies have revealed that each population of cholinergic neurons (particularly in the basal forebrain), catecholaminergic neurons, neuropeptide-containing neurons and nitric oxide-containing interneurons innervate the walls of cerebral arterioles (Edvinsson and Hamel 2002). These neurotransmitters/neuromodulators may control perfusion hemodynamics via the intimal cushion residing at the interior branching points of pial arterioles (Takayanagi et al. 1972; Edvinsson and Kraus 2002). The time lag between neuronal activity and blood flow change reflects the period required for controlling local blood flow. Spatial and temporal resolutions of TDD are thus often affected by such biological conditions. The sluggish hemodynamics obviously makes it difficult to distinguish movement-related BFV changes from instruction signal-related ones. Therefore, it should be mentioned here that simple average of signals during the movement period is not necessarily valid for obtaining signals related to movements only. This is also the case with other techniques for measuring hemodynamic responses. In the present study, however, the movement-related BFV changes are indeed represented by the difference between MI changes during the performance of the contralateral task versus the ipsilateral task.

The distance between the region of interest on color Doppler imaging and the acoustic transducer interferes with
spatial and temporal resolutions. Ultrasound released from the acoustic transducer is gradually weakened by absorption and scattering and is thus more easily absorbed, particularly at higher frequencies. In addition, obstructive ultrasound waves reflected from the lateral walls and base of the skull (i.e., nonremoved portions) to form acoustic artifacts (Fig. 3). Deeper structures in the brain thus display poorer resolution under color Doppler imaging. On the other hand, BFV changes observed under pulsed-wave Doppler mode can minimize the influence of distance between the sampling gate and acoustic transducer by adjusting Doppler angle and the Wall filter. Moreover, BFV changes recorded in the present experiments were normalized to the baseline during the control period. BFV changes in various cortical areas, including the MI, SMA, and PMv, are thus expected to prove reliable.

Functional Considerations
Accumulated evidence indicates that the SMA is involved in the execution of complex motor actions, such as bimanual coordination (Tanji et al. 1988; Matsuzaka et al. 1992; Kermadi et al. 1998; Ohara et al. 2000; Ullen et al. 2003; for review, see Tanji 1994, 2001). In favor of these electrophysiological and functional-imaging data from humans and nonhuman primates, the results of our TDD imaging clearly indicate that BFV increases in the SMA occur in relation to all of the bilateral, contralateral- and ipsilateral-movement tasks. However, BFV in the MI increases in response to bilateral and contralateral movements, but not to ipsilateral movement. Moreover, the SMA has been implicated in higher-order cognitive functions, including motor learning (Aizawa et al. 1991; Jenkins et al. 1994; Tanji and Shima 1994; Grafton et al. 2000; Lee and Quessy 2003; Halsband and Lange 2006). The present study revealed that SMA activity gradually decreases during the improvement of task performance, leaving MI activity unchanged. This implies that the SMA plays a crucial role in the acquisition of movements, particularly in the early stage, whereas the MI is related to simple motor execution. In addition, BFV changes in error trials were significantly lower in the SMA than those in success trials. This observation suggests that sufficient activation of the SMA during motor preparation by instruction signals may be necessary for proper execution of movements.

According to Shima and Tanji (2000), both the pre-SMA and SMA participate in sequential multiple movements. They reported that some SMA neurons display sequence-selective activity in particular sequence, but do not respond to the movement itself. In the present study, the complex discrimination task required the monkey to select one type from the 3 types of movements, whereas the simple movement task did not. Our results have shown that BFV changes in the SMA during the performance of the discrimination task are significantly larger than those in relation to the simple task, whereas such activity changes are not clearly detected in the MI. This suggests that the SMA may play an important role in motor selection, which favors the electrophysiological findings cited above.

Previous single-unit recording studies have shown that several motor-related areas in the frontal lobe—the pre-SMA (Matsuzaka et al. 1992; Hoshi and Tanji 2004), SMA (Mushiake et al. 1991; Kermadi et al. 1998; Russo et al. 2002), CMAd (Tanji et al. 2002; Isomura et al. 2003; Hoshi et al. 2005) and PMd/MDr (Fujii et al. 2000)—exhibit instruction signal-related activity during the performance of higher-order cognitive tasks. Our analysis using TDD imaging demonstrated that all of these motor-related areas responded strongly to instruction signals. On the other hand, many caudally situated motor-related areas, including the MI, CMAd, and PMv, display movement-related activity more prominently than instruction signal-related activity. It should be mentioned here, however, that the SMA is active in response to not only the instruction signal, but also the movement. These data indicate the validity of the TDD technique.

Intense BFV changes related to movement were observed mainly in the anterior bank of the central sulcus (Fig. 11), corresponding to the distal region of the MI, as shown in a previous intracortical microstimulation study with Japanese monkeys (Tokuno and Nambu 2000). During the performance of the present tasks, monkeys indeed used the wrist and digit only, with the shoulder and elbow restrained.

The present results have revealed that the pattern of BFV changes detected with TDD recording is in accordance with that of neuronal activity based on field-potential recordings. Furthermore, consistent with data obtained in previous studies (Tanji et al. 1988; Matsuzaka et al. 1992; Kermadi et al. 1998; Ohara et al. 2000; Ullen et al. 2003; for review, see Tanji 1994, 2001), the results of TDD recording clearly indicate involvement of the SMA in bilateral hand movements. Taken together, the TDD is useful for preliminary exploration, in advance of electrophysiological investigations, of brain regions related to particular functions. Moreover, TDD allows continual monitoring of changes in neuronal activity during the process of task training. Development of a more sensitive acoustic transducer with finer active matrix may permit noninvasive higher-resolution Doppler imaging.

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Notes
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