Distinct neuronal organizations of the caudal cingulate motor area and supplementary motor area in monkeys for ipsilateral and contralateral hand movements

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Nakayama Y, Yokoyama O, Hoshi E. Distinct neuronal organizations of the caudal cingulate motor area and supplementary motor area in monkeys for ipsilateral and contralateral hand movements. J Neurophysiol 113: 2845–2858, 2015. First published February 25, 2015; doi:10.1152/jn.00854.2014.—The caudal cingulate motor area (CMAc) and the supplementary motor area (SMA) play important roles in movement execution. The present study aimed to characterize the functional organization of these regions during movement by investigating laterality representations in the CMAc and SMA of monkeys via an examination of neuronal activity during a button press movement with either the right or left hand. Three types of movement-related neuronal activity were observed: 1) with only the contralateral hand, 2) with only the ipsilateral hand, and 3) with either hand. Neurons in the CMAc represented contralateral and ipsilateral hand movements to the same degree, whereas neuronal representations in the SMA were biased toward contralateral hand movement. Furthermore, recording neuronal activities using a linear-array multicontact electrode with 24 contacts spaced 150 μm apart allowed us to analyze the spatial distribution of neurons exhibiting particular hand preferences at the submillimeter scale. The CMAc and SMA displayed distinct microarchitectural organizations. The contralateral, ipsilateral, and bilateral CMAc neurons were distributed homogeneously, whereas SMA neurons exhibiting identical hand preferences tended to cluster. These findings indicate that the CMAc, which is functionally organized in a less structured manner than the SMA is, controls contralateral and ipsilateral hand movements in a counterbalanced fashion, whereas the SMA, which is more structured, preferentially controls contralateral hand movements.

macaque; bimanual movement; linear-array multicontact electrode; microarchitecture

FOUR MOTOR AREAS have been identified in the medial aspect of the primate frontal cortex: the supplementary motor area (SMA), the presupplementary motor area (pre-SMA), the rostral cingulate motor area (CMAr), and the caudal cingulate motor area (CMAc) (Amiez and Petrides 2014; Dum and Strick 1991; Matelli et al. 1991; Picard and Strick 1996; Tanji 1996; Vogt 2009; Vogt et al. 1987). The pre-SMA and CMAr are only sparsely interconnected with the primary motor cortex (M1) and the spinal cord (Hatanaka et al. 2003; Lu et al. 1994; Shima et al. 1991; Wang et al. 2001, 2004), but the SMA and CMAc exhibit strong interconnections with M1 (Hatanaka et al. 2003; Lu et al. 1994; Morecraft and Van Hoesen 1992; Shima et al. 1991; Wang et al. 2001) and the spinal cord (Dum and Strick 1991; He et al. 1995; Hutchins et al. 1988; Maier et al. 2002). This suggests that the CMAc and SMA are located hierarchically closer to the output stage of motor control. In fact, studies of single-cell activity in monkeys have revealed that both the CMAc and SMA are involved in the generation of arm movements (Backus et al. 2001; Cadoret and Smith 1997; Crutcher et al. 2004; Nachev et al. 2008; Nakajima et al. 2013; Paus 2001; Picard and Strick 1996; Shima et al. 1991; Shima and Tanji 1998; Tanji 1994).

A number of studies have assessed the response properties of neurons in the SMA. For example, Kermadi et al. (1998, 2000) demonstrated that neuronal activities in the CMAc and SMA are similarly modulated during unilateral and bilateral arm movements, and Russo et al. (2002) found that neurons in these regions exhibit vision-, set-, and movement-related activities. However, although it is evident that the directional selectivity of movement is greater in the SMA than in the CMAc (Richardson et al. 2008), the specific functional roles played by the CMAc and SMA remain largely elusive.

In human neuroimaging studies, the CMAc and SMA are active when subjects move either the ipsilateral or the contralateral hand (Babiloni et al. 2003; Baraldi et al. 1999; Diedrichsen et al. 2006; Immisch et al. 2001; Kollias et al. 2001; Naito et al. 2007; Roland et al. 1980). This suggests that the CMAc and SMA of a single hemisphere are involved with the movement of either hand. Similarly, single-cell studies in monkeys have shown that neurons in the SMA are active when subjects pull a lever with either arm (Brinkman and Porter 1979) and that the SMA is involved in the planning and execution of both single and bimanual hand movements in subjects trained to press a small button with their right hand, left hand, or both hands together (Tanji et al. 1987, 1988). However, to date, no studies have investigated the involvement of individual CMAc neurons in hand movements. Thus the present study examined neuronal activity in the CMAc and SMA of monkeys during the performance of a button press movement with either the right or left hand.

The present study had two major findings. First, an analysis of neuronal response selectivity revealed that the CMAc represents contralateral and ipsilateral hand movements in a counterbalanced manner to a greater extent than does the SMA. Second, an analysis of the spatial distribution of selective neurons within a small volume (spatial resolution: ~150 μm) revealed that CMAc neurons selective for the movement of either hand or both hands in this region are intermingled.
MATERIALS AND METHODS

Animals and appuratus. Two male monkeys (Macaca fuscata) weighing 8.0 kg (monkey 1) and 10.0 kg (monkey 2) were used in the present study. They were cared for as prescribed by the National Institutes of Health guidelines and the guidelines of the Tokyo Metropolitan Institute of Medical Science. All animal care and experimental procedures were approved by the Animal Care and Use Committee of Tokyo Metropolitan Institute of Medical Science. During the experimental sessions, each monkey sat in a primate chair with its head restrained and with small push buttons (23 mm in diameter) beneath each hand. Both forelimbs were comfortably restrained with straps, and the monkey could easily press the buttons using only hand movement. A 19-in. LCD monitor was placed 22 cm in front of the monkey, and its eye positions were monitored at 240 Hz with an infrared eye-tracking system (resolution: 0.25° visual angle; RHS-M; Applied Science Laboratories, Bedford, MA). The TEMPONET system (Reflective Computing, Olympia, WA) was used to control the behavioral task and the opening and closing of a solenoid valve serially installed in the reward delivery system.

Surgery. Before the initiation of physiological recording, anesthesia was induced in the monkeys with ketamine hydrochloride (10 mg/kg im) and atropine sulfate. The monkeys then underwent aseptic surgery while anesthetized with pentobarbital sodium (20-25 mg/kg iv), and antibiotics and analgesics were used to prevent infection and pain. During the surgery, polycarbonate and titanium screws were implanted in the skulls of the monkeys, and two plastic pipes were rigidly attached with acrylic resin. A section of the skull of each monkey over the left frontal lobe was removed, and a recording chamber was implanted to permit access to the CMAc and SMA.

Behavioral paradigm. The monkeys were trained to press a small button using the right or left hand or to press both buttons using both hands according to visual go signals on the LCD display (Fig. 1A). A trial was initiated when the monkey gazed at a fixation point (white circle, 1.4° visual angle), and if the monkey continued to fixate on this point for 500 ms, it disappeared and a visual go signal (white square, 2.7°) was presented in conjunction with a 1,000-Hz tone. The visual go signal, which appeared to the right, to the left, or on both sides of the fixation point (4.1° from center), instructed the monkey to press the right button, left button, or both buttons, respectively. Furthermore, the go signals were presented either once (i.e., requiring a single movement) or twice (i.e., requiring two movements). In the trials requiring two movements, each of the first and second go signals was randomly selected from the three movement types, resulting in nine combinations of the first and second movements. Of these trial types, the present study focused on trials in which a single movement of either the left or right hand was required.

The monkey was required to respond promptly, i.e., within 3.0 s, after each visual go signal with the tone was presented. During the single-movement trials, a correct response resulted in immediate removal of the visual go signal and the tone, followed 650 ms later by delivery of a drop of apple juice as a reward; the reward was followed by an intertrial interval of ≥3 s. If there was an incorrect response (pressing the button opposite to the go signal) or the monkey simultaneously pressed the right and left buttons (within 150 ms of each other), the visual go signal immediately disappeared, and a low tone (153 Hz) was presented for 0.5 s to indicate that the monkey had made an error. In this case, an intertrial interval of ≥3 s occurred without any reward. Identical visual go signals were presented successively in a block.

In addition to the trials in which the visual go signals were presented with the tone (visible condition), 60% of trials in the latter part of each block (>10 successful trials) were presented with the 1,000-Hz tone (auditory go signal) in the absence of a visual go signal (invisible condition). Under the invisible condition, the monkeys were still able to perform the task because the identical movement had been previously executed in that particular block of trials. In total, in >90% of cases, each block consisted of 13–21 trials. When each block ended, 5 bursts of the 1,000-Hz tone were presented to indicate that a new block of trials would follow. The types of movement in the subsequent blocks were selected randomly.

Physiological recording. All neuronal activity was recorded with a linear-array multi-contact electrode that had 24 contacts with a spacing of 150 μm (Plexon, Dallas, TX). The electrode was inserted into the brain through a 25-gauge guide tube that penetrated the dura mater under the power of a hydraulic microdrive (MO-971; Narishige, Tokyo, Japan) that moved the electrode in micrometer steps. To record neural activity in the SMA, the guide tube and electrode were inclined at a 10° angle lateral to the vertical axis to avoid bleeding from the superior sagittal sinus. To record neural activity in the CMAc, the guide tube and electrode were set vertically in the dorsoventral direction. All neuronal activity from the 24 contacts was simultaneously recorded, amplified, filtered (band-pass filter: 500 Hz to 8 kHz), digitized (sampling rate: 48,828 Hz; PZ2 and RZ2; Tucker-Davis Technologies, Alachua, FL), and saved on a laboratory computer (2210; Hewlett-Packard, Palo Alto, CA). Additionally, electromyographic (EMG) activity was recorded at 1,000 Hz with pairs of single-stranded Teflon-coated stainless steel wires (0.0762 mm in diameter; A-M Systems, Sequim, WA) that were inserted percutaneously. All EMG activity was amplified and digitized with an analog-to-digital converter, and the digital values were stored on the laboratory computer.

Neuronal activity was recorded in the left CMAc and SMA of the two monkeys. To reveal the somatotopic organizations of the CMAc and SMA, the present study assessed the neuronal responses evoked by somatosensory stimuli and the body movements produced by intracranial microstimulation (ICMS) applied through the tip of glass-coated tungsten electrodes inserted into these brain regions (44 cathodal pulses of 200 μs at 333 Hz, ≤80 μA; Luppino et al. 1993; Matsuzaka et al. 1992; Mitz and Wise 1987; Richardson et al. 2008; Shimaya et al. 2001; Takada et al. 2001). The recording sites were verified by examining magnetic resonance (MR) images (3.0T Magnetom Trio; Siemens, Erlangen, Germany; Fig. 1C).

Analysis of neuronal activity. Single-unit potentials were sorted offline using a spike-sorting software package (OpenSorter; Tucker-Davis Technologies), and all analyses of neuronal activity were performed using custom-made programs on MATLAB R2012b (The MathWorks, Natick, MA) and R version 3.0.2 software (R Foundation for Statistical Computing, Vienna, Austria).

We first counted the number of spikes from each neuron in successive 200-ms bins around two task events: go signal onset (5 bins: 3 before and 2 after onset) and movement onset (3 bins: 1 before and 2 after onset). We defined a neuron as “task related” if the distribution of the discharge rate (in spikes/s) differed significantly in at least one of the two trial types (right press or left press) as revealed by one-way ANOVA using the eight 200-ms bins as independent variables (α = 0.005). The numbers of spikes from each task-related neuron were counted during the baseline period (−500 to −300 ms relative to the onset of the visual go signal) and the movement period (−200 to 0 ms relative to the button press). Under the visible condition, neuronal activity in the baseline period was compared with neuronal activity in the movement period for each of the right-press and left-press trials (paired t-test, α = 0.01, uncorrected). Neuronal activity was classified as movement related if there was a significant difference in the number of spikes between the baseline period and the movement period in either type of trial. Subsequently, movement-related neurons were classified into three categories: 1) contralateral neurons (activity changes observed only during right-press trials), 2) ipsilateral neurons (activity changes observed only during left-press trials), and 3) bilateral neurons (activity changes observed during both right- and left-press trials). All movement-related responses were further analyzed to determine whether they were associated with increased or decreased activity. To accomplish this, the preferred side for each movement-related neuron (right press or left press associated
Fig. 1. Behavioral task, muscle activity data, and recording sites. A: temporal sequence of the behavioral task in which monkeys were required to press a button with their left (top) or right hand (bottom). B: examples of electromyographic (EMG) activity shown in the flexor carpi ulnaris (FCU) muscles of the left and right arms, respectively. Activities in the left-press trials (orange) and right-press trials (green) are aligned with the performance of the button press. EMG activity was smoothed with a boxcar average of 11 time bins (i.e., 11 ms), and the light blue dashed lines indicate the first of 10 consecutive bins (10 ms) that exhibited a significant difference between the left-press and right-press trials (Welch’s t-test, p < 0.01). The tick marks on the horizontal axis are placed at 200-ms intervals.

C: left and middle: top views of the surfaces of the left frontal cortices of the 2 monkeys. The orange circles indicate the sites of penetration where the caudal cingulate motor area (CMAc) neurons were recorded; the electrode was advanced vertically in the dorsoventral direction. The green circles indicate the sites of penetration where the supplementary motor area (SMA) neurons were recorded; the electrode was inclined at a 10° angle lateral to the vertical axis. Open squares indicate sites where intracortical microstimulation (ICMS) was used to evoke movement (44 cathodal pulses, 200-μs width at 333 Hz, ≤80 μA) or where neurons responded to somatosensory stimuli in the CMAc (red) or SMA (blue); the corresponding body parts are labeled. The light blue dashed lines and numbers indicate the distance (mm) anterior to the Horsley-Clarke interaural plane.

Right, coronal magnetic resonance images taken +17, +20, and +23 mm anterior to the Horsley-Clarke interaural plane in monkey 2. The thick lines indicate sites where electrodes penetrated the CMAc (orange) and SMA (green). Scale bars, 10 mm.

L, left hemisphere; R, right hemisphere. Schematic at bottom right shows where neurons were recorded in the CMAc (orange) and SMA (green).
with a greater change in activity) was determined, and if the activity of the preferred side was greater (or smaller) in the movement period than in the baseline period, then the neuron was defined as exhibiting increased (or decreased) activity.

A laterality index was calculated to quantify the laterality representation of the right or left hand:

$$\text{Laterality Index} = \frac{|FR_{\text{contra}}| - |FR_{\text{ipsi}}|}{|FR_{\text{contra}}| + |FR_{\text{ipsi}}|},$$

where $FR_{\text{contra}}$ refers to the difference in firing rates between the baseline period and the movement period for the right-press trials (i.e., the hand contralateral to the left hemisphere from which the neurons were recorded), and $FR_{\text{ipsi}}$ refers to this difference for the left-press trials (i.e., the hand ipsilateral to the left hemisphere from which the neurons were recorded). This index ranged from $-1$ to $+1$ and provided information regarding the extent of selectivity (with greater the absolute value indicating greater selectivity) and laterality (positive for contralateral preference and negative for ipsilateral preference).

**RESULTS**

**Muscle activity.** EMG activity was recorded from the forelimbs, neck, and paravertebral muscles. The deltoid, trapezius, supraspinatus, infraspinatus, biceps, triceps, neck, and paravertebral muscles did not exhibit consistent changes in activity relative to movement execution. In contrast, the flexor carpi ulnaris (FCU), which is the primary muscle involved in the hand-only button press movement, did exhibit changes in activity that were associated with movement execution (Fig. 1B). More specifically, in the limb required to move, the FCU exhibited phasic bursts of activity during movement execution, whereas the FCU in the limb not required to move did not exhibit phasic changes in activity. These findings indicate that the monkeys executed the button press using hand movement on only the required side.

We calculated the onset time of the FCU activity in relation to the completion of the button press for each monkey by identifying the first of 10 consecutive 1-ms bins (10 ms) that exhibited a significant difference between the left-press and right-press trials (Welch’s $t$-test, $\alpha = 0.01$). The FCU onset times of ipsilateral hand movement were $-115$ ms for monkey 1 and $-94$ ms for monkey 2; those of contralateral hand movement were $-135$ ms for monkey 1 and $-107$ ms for monkey 2 (Fig. 1B).

**Recording of neuronal activity in the CMAc and SMA.** During task performance, neuronal activities were recorded using a linear-array multicontact electrode inserted into the CMAc or SMA (Fig. 1C). Neurons in the SMA were recorded from medial wall of the frontal cortex, and those in the CMAc were recorded from the dorsal and ventral banks of the cingulate sulcus. The recording sites in the CMAc were confined to lateral aspects of the banks of the cingulate sulcus, and the most medial aspects were not explored. In separate sessions, the tungsten microelectrode (rather than the multicontact electrode) was employed to identify the somatotopic organizations of the CMAc and SMA, which were identified using movements evoked by ICMS or by recording neuronal responses to somatosensory stimuli applied by the experimenter. The present study observed neuronal spikes in and around the forelimbs, neck, and paravertebral regions of the CMAc and SMA (Fig. 1C).

The present study primarily focused on neural activity recorded during successful trials because the success rate for both monkeys exceeded 94% (98.1% in monkey 1 and 94.5% in monkey 2). The CMAc and SMA neurons were sampled during at least two blocks of each of the right-press and left-press trials. We found three classes of neurons in these areas. The activity of contralateral neurons changed only in association with right-handed button presses, i.e., contralateral to the recorded hemisphere. Figure 2A shows an example of a contralateral neuron that exhibited increased activity during
button pressing with the right (contralateral), but not the left (ipsilateral), hand. The activity of ipsilateral neurons changed only in association with left-handed button presses, i.e., ipsilateral to the recorded hemisphere. Figure 2B shows an example of an ipsilateral neuron that exhibited increased activity during button pressing with the ipsilateral, but not the contralateral, hand. The activity of bilateral neurons changed in association with both contralateral and ipsilateral button pressing. Figure 2C shows an example of a bilateral neuron that exhibited increased activity during button pressing with either the ipsilateral or contralateral hand. All three classes of neurons were found in both the CMAc and SMA, indicating that these areas are involved in movement of either hand.

Population neuronal activity in the CMAc and SMA. In total, we sampled the activity of 970 CMAc neurons (659 in monkey 1 and 311 in monkey 2) and 525 SMA neurons (356 in monkey 1 and 169 in monkey 2). Of these, 891 CMAc neurons and 476 SMA neurons exhibited task-related activity. To explore the overall hand representations of these task-related neurons, we sampled the activity of 970 CMAc neurons (659 in monkey 1 and 311 in monkey 2) and 345 SMA neurons (236 in monkey 1 and 109 in monkey 2) for the right-press trials and at 130 ms in the left-press trials. In both areas, the population activity was greatest in relation to movement execution. The activity peaked just before completion of the button press. In the CMAc (Fig. 3A), the peaks occurred at −70 ms in the right-press trials and at −60 ms in the left-press trials relative to the button press. In the SMA (Fig. 3B), they occurred at −110 ms in the right-press trials and at −130 ms in the left-press trials. In the CMAc, the population activity did not differ between the right-press and left-press trials except for 6 of the 120 10-ms bins [−600 to 600 ms relative to the button press; a paired t-test (α = 0.01) was used to compare the activity of successive bins measured by the inverse-interspike interval method; Hoshi and Tanji 2006]. In contrast, in the SMA, the population activity for the right-press trials was greater than that for the left-press trials. In 34 of the 120 10-ms bins, the activity was greatest in the right-press trials than the left-press trials.
findings indicate that SMA neurons with increased activity preferentially represented contralateral hand movement, whereas CMAc neurons with increased activity represented contralateral and ipsilateral hand movements in a counterbalanced manner. Furthermore, neurons with decreased activity in both the CMAc and SMA represented the movements of either hand in a counterbalanced manner.

For each neuron, changes in activity during the movement period relative to the baseline period were calculated separately for the right-press and left-press trials. These data were used to plot the activity modulation for each neuron in the CMAc (Fig. 5A) and SMA (Fig. 5B), revealing that contralateral neurons were clustered around the x-axis and ipsilateral neurons were grouped around the y-axis, consistent with the notion that activity modulation in the contralateral (ipsilateral) neurons was greater during contralateral (ipsilateral) hand movement. In contrast, bilateral neurons were clustered around the line-of-unity slope, which indicates that the direction of activity modulation (increase/decrease) in these neurons was similar between the right-press and left-press trials.

For each neuron, the laterality index was calculated to quantify the representations for contralateral and ipsilateral hand movements (see MATERIALS AND METHODS). The mean value of the laterality indexes in the CMAc (Fig. 5C) was not significantly different from zero (mean = 0.0097; one-sample t-test: $t = 0.4582$, $P = 0.6470$), whereas that of the laterality indexes in the SMA (Fig. 5D) was significantly greater than zero (mean = 0.1414; one-sample t-test: $t = 5.1548$, $P < 0.0001$). To further explore the laterality representation, we divided all neurons into those exhibiting contralateral and those exhibiting ipsilateral preferences on the basis of the signs of the laterality indexes. In the CMAc (Fig. 5E), the cumulative distributions of the absolute values of the laterality indexes did not differ between contralateral-prefering neurons (neurons with positive laterality index values, $n = 282$, median = 0.3712) and ipsilateral-prefering neurons (neurons with negative laterality index values, $n = 282$, median = 0.3712).

Fig. 5. Selectivity of neuronal activity for right-press and left-press movements. A and B: relationships of the changes in firing rates between the right-press and left-press trials in the CMAc (A) and SMA (B). The changes in movement-related firing rates from the baseline period for the right-press trials are plotted along the abscissa, and those for the left-press trials are plotted along the ordinate. The positive and negative values denote increased and decreased activity, respectively. The area of each pie chart is proportional to the total number of neurons in the CMAc and SMA; the percentage and actual numbers of neurons in each category are shown next to the corresponding portion of each pie chart. Each category is color coded: red, contralateral neurons; blue, ipsilateral neurons; and gray, bilateral neurons.

Fig. 4. Frequencies of neurons selective for contralateral, ipsilateral, or bilateral movements. The pie charts summarize the proportions of neurons in the CMAc (A) and SMA (B) that were classified into each of the three categories. Left, the classification of all movement-related neurons; middle and right, the classifications of the neurons associated with increased and decreased activity, respectively. The area of each pie chart is proportional to the total number of neurons in the CMAc and SMA; the percentage and actual numbers of neurons in each category are shown next to the corresponding portion of each pie chart. Each category is color coded: red, contralateral neurons; blue, ipsilateral neurons; and gray, bilateral neurons.
FUNCTIONAL ORGANIZATION OF CMAc AND SMA FOR HAND MOVEMENT

A. Firing Rate Change (CMAc)

B. Firing Rate Change (SMA)

C. Magnitude of Laterality (CMAc)

D. Magnitude of Laterality (SMA)

E. Cumulative Fraction of Laterality (CMAc, all neurons)

F. Cumulative Fraction of Laterality (SMA, all neurons)

G. Cumulative Fraction of Laterality (CMAc, classified)

H. Cumulative Fraction of Laterality (SMA, classified)
Table 1. Peak and trough times of neurons

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Values are peak and trough times (ms) of neurons in the caudal cingulate motor area (CMAc) and supplementary motor area (SMA) of monkeys during left-press or right-press trials.
Fig. 6. Time courses of the population selectivity. A–H: population activities are shown for CMAc neurons exhibiting increased activity (A) and decreased activity (B) and for SMA neurons exhibiting increased activity (C) and decreased activity (D). The mean firing rate (±SE; bin width = 10 ms) is aligned with the button press separately for right-press trials (green) and left-press trials (orange); left, contralateral neurons; middle, ipsilateral neurons; and right, bilateral neurons. Population selectivity is shown for CMAc neurons exhibiting increased activity (E) and decreased activity (F) and for SMA neurons exhibiting increased activity (G) and decreased activity (H). The population selectivity (bin width = 10 ms) was calculated by subtracting activity in the left-press trials from activity in the right-press trials. The selectivity is aligned with the button press; red, contralateral neurons, blue, ipsilateral neurons, and black, bilateral neurons. The red and blue translucent areas indicate 10-ms bins in which selectivity was significantly different from zero for the contralateral and ipsilateral neurons, respectively (1-sample t-test, p < 0.01). Arrows indicate the first of 5 consecutive bins that exhibited significant selectivity.

I and J: cumulative distributions of onset time for individual neurons in the CMAc (I) and SMA (J). Magenta solid line, contralateral neurons for right-press trials; cyan solid line, ipsilateral neurons for left-press trials; magenta dashed line, bilateral neurons for right-press trials; cyan dashed line, bilateral neurons for left-press trials.
neurons, the proportions of counterpart neurons with the same selectivity as the reference neurons were greater than the expected values (contralateral neurons: 48%, P = 0.0091; ipsilateral neurons: 38%, P = 0.0021; bilateral neurons: 25.3958, P = 0.0001). A post hoc residual analysis (chi-square test: \( \chi^2 = 5.2917, P = 0.0258; \) Fig. 7C). On the other hand, the frequencies of the contralateral, ipsilateral, and bilateral counterpart neurons in the SMA differed significantly depending on the selectivity of the reference neurons (chi-square test: \( \chi^2 = 25.3958, P < 0.0001; \) Fig. 7D). A post hoc residual analysis (Haberman 1973) applied to the SMA neurons revealed that for each of the contralateral, ipsilateral, and bilateral reference neurons, the proportions of counterpart neurons with the same selectivity as the reference neurons were greater than the expected values (contralateral neurons: 48%, P = 0.0091; ipsilateral neurons: 38%, P = 0.0021; bilateral neurons: 25.3958, P = 0.0001). The distribution of hand selectivity within a small cortical volume was assessed by comparing the selectivity of a pair of simultaneously recorded neurons. We first identified a reference neuron that 1) exhibited any hand selectivity and 2) had an adjacent contact with a cell that exhibited any hand selectivity. In this manner, 135 contralateral, 123 ipsilateral, and 167 bilateral CMAc neurons and 71 contralateral, 42 ipsilateral, and 101 bilateral SMA neurons were identified as reference neurons. Next, the distribution of selectivity in the counterpart neurons that were recorded simultaneously with the reference neurons for the contralateral (left), ipsilateral (middle), and bilateral (right) hand movements. The frequencies of the contralateral, ipsilateral, and bilateral counterpart neurons in the CMAc did not significantly differ by the selectivity of the reference neurons (chi-square test: \( \chi^2 = 5.2917, P = 0.0258; \) Fig. 7C). On the other hand, the frequencies of the contralateral, ipsilateral, and bilateral counterpart neurons in the SMA differed significantly depending on the selectivity of the reference neurons (chi-square test: \( \chi^2 = 25.3958, P < 0.0001; \) Fig. 7D). 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ipsilateral neurons: 47%, \( P < 0.0001 \); and bilateral neurons: 56%, \( P = 0.0048 \); Fig. 7D). This finding indicates that neurons in the SMA with identical selectivity tended to form clusters, which is evident in the three SMA sample recordings (Fig. 7B).

In the same manner, the selectivity distributions of the counterpart neurons were analyzed at 300 and 450 \( \mu \text{m} \). A reference neuron was identified where 1) a neuron exhibited any hand selectivity and 2) a contact located at 300 (for the 300-\( \mu \text{m} \) analysis) or 450 \( \mu \text{m} \) (for the 450-\( \mu \text{m} \) analysis) distant from the contact with the reference neuron involved a cell that exhibited any hand selectivity. In the CMAc, the selectivity distributions of counterpart neurons at 300 or 450 \( \mu \text{m} \) did not differ with respect to the selectivity of reference neurons (chi-square test: \( P \geq 0.01 \); Fig. 7C). Next, the distributions of neurons in the SMA were assessed. Those of the counterpart SMA neurons at 300 \( \mu \text{m} \) significantly differed depending on the selectivity of the reference neurons (chi-square test: \( \chi^2 = 23.6539, P < 0.0001 \)). Additionally, a post hoc residual analysis showed that neurons with an identical selectivity tended to form a cluster (contralateral neurons: 54%, \( n = 42, P = 0.0002 \); ipsilateral neurons: 39%, \( n = 16, P = 0.0005 \); and bilateral neurons: 54%, \( n = 48, P = 0.0050 \)). In contrast, the selectivity distributions of the counterpart SMA neurons at 450 \( \mu \text{m} \) did not significantly differ depending on the selectivity of the reference neurons (chi-square test: \( \chi^2 = 2.2025, P = 0.6986 \)). These findings indicate that the radii of the clusters of the contralateral, ipsilateral, and bilateral SMA neurons were \(<450 \mu \text{m} \).

Although the above analyses revealed that the neuronal distributions of the SMA and CMAc were significantly different, this contrast may have been derived from differences in the electrode penetration within the cortex. For example, the contacts in the SMA were arranged in a manner more parallel to the cortical surface of the brain than were the contacts in the CMAc because electrodes in the CMAc were more vertically oriented than those in the SMA. To assess the impact of electrode angle, the distributions of the CMAc neurons that were recorded from penetrations that were \( \leq 0.5 \text{ mm} \) from the most lateral level were analyzed. Because this site corresponds to the fundus of the cingulate sulcus, these contacts were arranged less vertically to the cortical surface of the brain than were the electrodes that were placed more medially. Even in the lateral recordings from the CMAc, the frequencies of the contralateral, ipsilateral, and bilateral counterpart neurons (150 \( \mu \text{m} \) apart) did not differ depending on the selectivity of the reference neurons (chi-square test: \( \chi^2 = 2.4806, P = 0.6481 \)). This finding indicates that the distinct SMA and CMAc neuronal distributions did not result solely from the manner in which the contacts were arranged within the cortex but that the patterns of neuronal distributions within these regions were truly different.

**DISCUSSION**

In the present study, neuronal activity was recorded while the monkeys performed a button press task with either the right or left hand. Analyses of neuronal activity immediately before and during movement execution revealed that the CMAc represented contralateral, ipsilateral, and bilateral hand movements in a counterbalanced manner, whereas the SMA preferentially represented contralateral and bilateral hand movements with only minor representation of ipsilateral hand movement. Furthermore, an analysis of the spatial distributions of neuronal activity at a submillimeter scale revealed that the CMAc and SMA displayed distinct microarchitectural organizations. CMAc neurons representing contralateral and ipsilateral hand movements were homogeneously intermingled, whereas SMA neurons with identical hand preferences tended to aggregate.

**Laterality representations of movement-related activity in the CMAc.** Human studies have demonstrated that the caudal cingulate zone (CCZ), which is analogous to the CMAc in monkeys, is active regardless of which arm is used during task performance (Dinstein et al. 2008; Immisch et al. 2001; Kollias et al. 2001). This suggests that the CMAc may be involved during the execution of bilateral arm movements by monkeys. Kermadi et al. (2000) examined neuronal activity in the CMAc of monkeys performing reach and grasp movements and found that a vast majority of neurons (77%) were active during the movement of either arm (bilateral neurons). In contrast, the present study found that a smaller proportion (41%) of CMAc neurons were active during bilateral movements. This disparity may result from differences in the types of movements required by the different tasks; Kermadi et al. (2000) measured whole arm movements, whereas the present study assessed hand-only movements. The remaining neurons of the present study were selective for either right or left hand movements. The activity modulation of ipsilateral neurons did not differ from that of contralateral neurons, and the neuronal activity began before the initiation of the muscle activity. Together, the data presented in this report provide compelling evidence that the CMAc is not only involved in the general control of hand movements irrespective of which hand is used but also is actively engaged during specific control of contralateral and ipsilateral hand movements.

**Laterality representations of movement-related activity in the SMA.** Previous investigations of the laterality representations of movement execution in SMA neurons have measured whole arm movements (Brinkman and Porter 1979; Donchin et al. 1998, 2002; Kermadi et al. 1998). Similar to the laterality representations in CMAc neurons (Kermadi et al. 2000), a great majority of SMA neurons are classified as bilateral. Because whole arm movements are often accompanied by muscle activity in the nonperforming arm and inevitably include postural muscle activity, it is possible that bilateral neurons are overrepresented. To resolve this issue, Tanji et al. (1987, 1988) required monkeys to execute the button press action using hand-only movements. A reanalysis of the reported data from Tanji et al. (1987, 1988) revealed that 54%, 37%, and 9% of SMA neurons were classified as bilateral, contralateral, and ipsilateral neurons, respectively. The results of the present study are consistent with these findings in that more neurons were classified as contralateral than as ipsilateral. Additionally, the present study found that the activity modulation was biased toward the contralateral hand movement, indicating that the SMA preferentially represented the contralateral hand movement, although a substantial number of neurons also represented ipsilateral hand movement.

**Temporal profiles of movement-related activity.** We found that the activity and hand use selectivity of CMAc and SMA neurons commenced earlier than did the onset of muscle activity. This indicates that both the CMAc and SMA are involved in the genesis of hand movement. We also found that
the onset time of movement-related activity of the contralateral, ipsilateral, and bilateral neurons was similar in the CMAc (Fig. 6I) and SMA (Fig. 6J). Moreover, the duration of movement-related activity of these neurons did not differ between the two brain areas. These findings suggest that movement-related activity in the CMAc and SMA begin simultaneously and are of comparable duration. However, the timing of activity peaks differed between the CMAc and SMA. The activity of all task-related neurons in the SMA peaked earlier than that in the CMAc (Fig. 3 and Table 1). In the contralateral, ipsilateral, and bilateral neurons, the peak (trough) times associated with increased (decreased) activity of SMA neurons were earlier than those of CMAc neurons (Fig. 6, A–H, and Table 1). These results indicate that the SMA consistently reaches activity peaks earlier than does the CMAc, raising the possibility that the SMA may play a leading role in commitment to action execution, whereas the CMAc may make a greater contribution to the actual execution of unfolding action.

Distinct microarchitectural organizations of the CMAc and SMA. We employed linear-array multicontact electrodes to simultaneously sample the activity of multiple neurons within a small cortical volume (several millimeters) (Bonini et al. 2014; Takeuchi et al. 2011). There are two major limitations to this method. First, the spatial resolution of neuronal recordings (~100 μm) is 10 times coarser than that obtained via in vivo imaging (~10 μm) (Komiyama et al. 2010; Sato et al. 2007). Second, neuronal sampling is linear or one dimensional, whereas two- or three-dimensional sampling is possible using in vivo imaging. However, our use of linear-array multicontact electrodes allowed us to explore neuronal organization in the brain of a nonhuman primate without introducing major changes to the recording system. Also, we were able to sample neurons from virtually any region of the brain, including mesial aspects and banks from which it is often difficult to obtain in vivo images; the monkey CMAc and SMA are typical examples of such tissues. Furthermore, direct recording of extracellular spikes allowed us to perform cross-correlation analysis between the spike trains of different neurons, yielding detailed data on information flow among neurons and computations performed within local circuits.

We could not monitor movement-related neurons from all successive contacts. Rather, movement-related neurons were sampled in clusters, in a rather sparse manner, as shown in Fig. 7, A and B. We offer two possible explanations for this sparseness. First, silent contacts may contain neurons encoding other movement types, such as wrist extension, reaching, or grasping, or neurons encoding movement of other body parts, such as the elbow or shoulder. Second, silent contacts may be too far away from actively discharging neurons to register electric activity.

However, our focus on the distributions of contacts adjacent to (within ±450 μm) reference neurons suggests that the microarchitecture of the CMAc may differ from that of the SMA in several respects. In the SMA, neurons with the same selectivity tended to cluster in a manner that was suggestive of spatial grouping, and the radii of the clusters of the contralateral, ipsilateral, and bilateral neurons were estimated to be <450 μm. In the CMAc, the proportions of neurons selective for contralateral (30%) and ipsilateral (29%) hand movements were comparable (Fig. 4A), and the contralateral, ipsilateral, and bilateral neurons were distributed homogeneously without any clustering.

It has been suggested that there are three protogradations (directions of progressive cortical differentiation) in the frontal cortex of primates (Goldberg 1985; Sanides 1964, 1970; Sanides and Krishnamurti 1967): 1) the lateral protogradation, which originates in the insular cortex; 2) the most recent evolutionary protogradation, which originates in the central sulcus; and 3) the medial protogradation, which originates in the cingulate gyrus. The CMAc is situated immediately next to the origin of the medial protogradation (the cingulate gyrus) and may be an evolutionally older aspect of the motor cortex. An intriguing possibility is that, much like the more homogenous cellular architecture found in evolutionarily older cortex (Barbas 1986; Barbas and Pandya 1987, 1989; Garcia-Cabezas and Barbas 2014; Shipp 2005), the evolutionally older CMAc may also employ a more homogenous functional architecture. If this is the case, evolutionarily newer motor areas may employ a more structured organization than that of the CMAc, as is observed in the SMA. Future studies that record neurons across all cortical layers with the use of higher spatial resolution (Hira et al. 2013) to investigate these possibilities may lead to a better understanding of the basic principles and mechanisms underlying the motor control that is realized by the neural circuitry in the motor areas of the frontal lobes (Cisek et al. 2003; Hoshi and Tanji 2007; Kalaska and Crammond 1992; Kurata 2010; Tanji 1994; Wise and Maurit 1985).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Y.N., O.Y., and E.H. conception and design of research; Y.N. performed experiments; Y.N., O.Y., and E.H. analyzed data; Y.N., O.Y., and E.H. interpreted results of experiments; Y.N. prepared figures; Y.N., O.Y., and E.H. drafted manuscript; Y.N., O.Y., and E.H. edited and revised manuscript; Y.N., O.Y., and E.H. approved final version of manuscript.

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