Articles in PresS. J Neurophysiol (September 20, 2017). doi:10.1152/jn.00279.2017

Movement-related activity in the periarcuate cortex of monkeys during coordinated eye and hand movements

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Running Head: Periarcuate cortex in coordinated eye and hand movements

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Text pages: 50

Abstract word count: 204

Words in New and Noteworthy: 75

Number of figures: 13

Number of tables: 2

ABSTRACT

To determine the role of the periarcuate cortex during coordinated eye and hand movements in monkeys, the present study examined neuronal activity in this region during movement with the hand, eyes, or both as effectors toward a visuospatial target. Similar to the primary motor cortex (M1), the dorsal premotor cortex contained a higher proportion of neurons that were closely related to hand movements, whereas saccade-related neurons were frequently recorded from the frontal eye field (FEF). Interestingly, neurons that exhibited activity related to both eye and hand movements were recorded most frequently in the ventral premotor cortex (PMv), located between the FEF and M1. Neuronal activity in the periarcuate cortex was highly modulated during coordinated movements compared to either eye or hand movement only. Additionally, a small number of neurons were active specifically during one of the three task modes, which could be dissociated from the effector activity. In this case, neuron onset was either ahead of or behind the onset of eye and/or hand movement, and some neuronal activity lasted until reward delivery signaled successful completion of reaching. The present findings indicate that the periarcuate cortex, particularly the PMv, plays important roles in orchestrating coordinated movements from the initiation to the termination of reaching.

NEW & NOTEWORTHY

We recorded movement-related neuronal activity throughout the periarcuate cortex of monkeys who performed a task requiring them to move their hand only, eyes only, or both hand and eyes toward visuospatial targets. Most typically, we found neurons that were commonly active regardless of different effectors, from movement initiation to completion of a successful outcome. We suggest that the periarcuate cortex as a whole plays a crucial role in initiating and completing coordinated eye-hand movements.

KEYWORDS

Eye-hand coordination, periarcuate cortex, reaching

INTRODUCTION

When human tennis players reach for and hit the ball, they must perform coordinated eye and hand movements to hit the ball accurately, and must then ascertain the outcome of the reaching action via visual and other sensory input. Similarly, nonhuman primates reach toward a target using coordinated eye and hand movements that involve visual guidance supported by least two processes in the brain. Prior to movement initiation, visuospatial information from the target is transformed into general and then specific motor commands for reaching movements. Once the movement is initiated, the subject usually continues to track the movement to ensure the outcome (Gordon and Ghez 1987; Todorov and Jordan 2002).

There are several cortical motor areas in the monkey brain, but the regions around the arcuate sulcus are most likely to be crucial for this type of movement behavior. Of the regions surrounding the arcuate sulcus, the ventral and dorsal premotor cortices (PMv and PMd, respectively), which are caudal to the arcuate sulcus (postarcuate cortex), play important roles in reaching movements with a hand in conjunction with eye position, but not necessarily eye movement (Cisek and Kalaska 2005; Hoshi and Tanji 2006; Kurata and Hoshi 2002; Pesaran et al. 2006). In contrast, the frontal eye field (FEF), which is rostral to the arcuate sulcus (prearcuate cortex), primarily supports preparation and initiation of saccadic eye movements (Bruce and Goldberg 1985; Schall 1991b) and eye fixation (Izawa et al. 2009). In addition to the FEF, which is located in area 8, neurons related to smooth pursuit and saccadic eye movements have been

identified in the deep aspect of the postarcuate cortex (MacAvoy et al. 1991; Tanaka and Fukushima 1998), which is referred to as the premotor eye field (Amiez and Petrides 2009).

However, little is known regarding the degree to which the three cortical areas around the arcuate sulcus are selectively involved in eye or hand movements toward a target or about how these adjacent regions contribute to the initiation, specification, execution, and completion of coordinated eye–hand movements toward a common target. Thus, the present study investigated whether the three cortical regions around the arcuate sulcus (PMv, PMd, and FEF) contribute to movements of the eyes, hands, or both by recording and comparing neuronal activity in these regions in monkeys that were trained to perform three tasks: (1) coordinated eye and hand movements toward a common target (Both task), (2) saccadic eye movements without hand movement (Eye task), and (3) hand movement without eye movement (Hand task). The present study focused on movement-related activity during initiation and completion of a reaching movement that was signaled as successful by delivery of a reward.

MATERIALS AND METHODS

Animals and apparatus

The present study included two Japanese monkeys (*Macaca fuscata*, weight: 5.1–6.4 kg) that were handled according to the Guide for the Care and Use of Laboratory Animals (National Research Council; Washington, DC) and the Guidelines for Handling Japanese Monkeys (Committee of National BioResearch Project, Japan). All experimental procedures were approved by the Animal Experimentation Committee of Hirosaki University.

The monkeys performed a behavioral task controlled by the TEMPO-NET system (Reflective Computing; Olympia, WA, USA). The same system was used to retrieve and store all behavioral, neuronal, and digitally converted analog data, including eye and hand positions and electromyographic (EMG) data sampled at 1 kHz. During the procedure, the monkeys sat comfortably in a primate chair facing a 19-in liquid crystal display (LCD-A193V, 1280 × 1024 pixels, I-O Data, Japan) placed 48 cm from the monkeys' eyes. The horizontal and vertical distances between the central holding zone and the center of each target were 11 cm on the tablet and 8° on the LCD. A computer mouse (WACOM Intuos2 2D mouse, Wacom Technology, Corp.; Vancouver, WA, USA) was attached to the right palm of each monkey using orthopedic elastic tape (Elasticon 75 mm, Johnson & Johnson; New Brunswick, NJ, USA); the location of the mouse was detected with a 457.2 × 304.8-mm digitizer (WACOM Intuos2 i-1820; Wacom Technology Corp.), sampled at 200 Hz with 10-μm resolution, and displayed on the LCD using a cross-shaped cursor (Fig. 1A).

An opaque barrier was placed between the monkeys and the digitizer so that the monkey was unable to see either its own hand or the mouse. During the experiment, the monkey's left hand was strapped to the primate chair. Eye movements were sampled at 250 Hz using an infrared oculometer (model R21C-A, RMS; Hirosaki, Japan), and the horizontal and vertical positions of the left eye were used to monitor eye movements throughout the task.

Behavioral task

The monkeys were comfortably seated in the primate chair and trained to perform the behavioral task using either their eyes, right hand, or both; the task sequence is detailed in Figure 1. A single small square in the center of the screen and four large squares equidistant from the central square were shown on the LCD throughout the session (Fig. 1A). The center of each peripheral target zone was indicated by a small stationary white cross, and the central and peripheral open squares served as the central holding zone and the target zones, respectively. The monkey's hand position was indicated by a large white cross corresponding to the position of the computer mouse being controlled by the monkey.

Monkeys initiated a trial by fixating on the central small square and holding the computer mouse in the central holding zone (first panel, Fig. 1A). Monkeys were required to maintain the position of their eyes and hands within the holding zone for a preparation period of 1.7–2.2 s, during which two visual instruction cues were presented at different times: (1) the effector instruction for the impending movement (eyes, hand, or both), which was pseudorandomly selected

and indicated by a green, red, or yellow square that replaced the gray central fixation square; and (2) the target instruction, which was indicated by a small white square at the center of a pseudorandomly selected peripheral target zone (second and third panels, Fig. 1A). Based on the effector to be used, the three conditions were referred to as the Eye, Hand, and Both trials; the two instruction signals were presented in pseudorandom order in each trial.

After the preparation period, the Go signal was presented by changing the color of the central fixation square to blue (fourth panel, Fig. 1A). The monkeys were required to initiate the movement using the required effector(s) within 500 ms of the presentation of the Go signal (fifth panel, Fig. 1A) and then acquire the target within 500 ms of movement onset (sixth panel, Fig. 1A). Movement initiation was detected when the eyes and/or hand left the central zone. In the Eye and the Both trials, monkeys were required to perform a saccade from the central fixation zone to a peripheral target and then maintain eye fixation on the target after acquiring it. In the Hand trials, monkeys were required to maintain eye fixation on the central fixation zone. In the Hand and Both trials, monkeys were required to move the hand from the central holding zone to reach toward the peripheral target, and then hold the hand position within the target zone after acquiring it. In the Eye trials, monkeys were required to hold the hand in the central holding zone. In the Both trials, eye and hand movement onsets were detected separately.

The time between the Go signal and saccade onset and between the Go signal and hand movement onset were termed the eye reaction time (RT) and hand

RT, respectively. Similarly, the interval between saccade onset and target acquisition and between hand movement onset and target acquisition were termed eye movement time (MT) and hand MT, respectively. If a monkey maintained its eye and hand positions within the required hold zones for 200–500 ms (seventh panel, Fig. 1A) after capturing the target, a drop of juice (0.08 mL) was delivered to reward a successful trial. Following the delivery of the reward, the monkeys were allowed to release the holding position at the target (eighth panel, Fig. 1A). If the monkey failed to maintain the required holding and target zones during the preparation periods or during the periods between target acquisition and reward delivery, the trial was aborted and restarted from the beginning.

Surgery and data acquisition

Following the completion of behavioral training, the monkeys were surgically prepared under aseptic conditions using nitrous oxide (50%) and isofluorothane (1–2%) anesthesia after induction with ketamine hydrochloride (8 mg/kg, intramuscular [i.m.]) and atropine sulfate. Four head-restraining bolts and one rectangular stainless steel recording chamber (27 × 27 mm) were implanted in the skull. The chamber was centered at 12.0 mm anterior and 18.0 mm lateral over the left hemisphere according to the Horsely–Clarke stereotaxic frame. Analgesics and antibiotics were applied to prevent postsurgical pain and infection.

After complete recovery from the surgery, neuronal activity was recorded using glass-insulated Elgiloy microelectrodes (1.0–1.5 MΩ at 333 Hz) inserted into the periarcuate cortex and primary motor cortex (M1) of the left hemisphere.

The microelectrode was driven by a hydraulic microdrive (MO95; Narishige; Tokyo, Japan). The electrode signals were amplified and filtered with a multichannel processor (MCP; Alpha-Omega Engineering, Nazareth, Israel) and sorted using a multi-spike detector (MSD, Alpha-Omega Engineering) simultaneously isolated three neurons. During neuronal recording, intracortical microstimulation (ICMS) was employed to identify the FEF and to determine the borders between M1, the PMd, and the PMv. If rapid eye movements were evoked by ICMS at less than cathodal 50 μA (333 Hz, 11 train pulses with a 0.2-ms pulse width) in the pre-arcuate cortex, then the area was defined as the FEF (Bruce and Goldberg 1985). If somatic movements were evoked by ICMS at less than 50 μA (same parameters as above) in the precentral cortex, then the area was defined as M1. The premotor cortex was defined as a location rostral to M1 in the precentral gyrus where no somatic movements were evoked using the abovementioned ICMS parameters (Kurata 1993; Weinrich and Wise 1982). The penetration locations were confirmed using standard histological techniques, including Nissl staining and electrolytic marking lesions. EMG activity was sampled bilaterally by placing wire electrodes in the anterior deltoid, trapezius, supraspinatus, infraspinatus, pectoralis major, rhomboid, thoracic paravertebral, biceps, and triceps brachii muscles. All EMG data were band-pass filtered between 20 Hz and 5 kHz and sampled at 1 kHz.

Data analysis

All data analyses, including for the neuronal data, were performed using Matlab 2015b with its statistical toolbox (MathWorks; Natick, MA, USA). First, the precise onsets of eye and hand movements were determined from the movement trajectories; then, neuronal activity related to eye movements (saccades and fixation) or hand movements was quantitatively analyzed. For these analyses, the instantaneous firing rate was converted from the interspike interval at a 1-ms resolution (Hoshi and Tanji 2006), and neuronal spike frequency data were aligned to onsets of hand and eye movement in the three trial types (Hand, Eye, and Both) in four directions (right, up, left, and down; Fig. 4). For data recorded in the Both trials, two types of raster displays were created by aligning the hand and eye onsets; thus, 16 raster displays and histograms were created for each neuron.

To define movement-related neuronal activity, the mean and standard deviation (SD) of the instantaneous firing rate at 250–750 ms before the presentation of the Go signal (pre-Go control period) were calculated. Then, this value was compared with the mean discharge rate of the same neuron during the period between presentation of the Go signal for movement initiation and reward delivery signaling the end of the trial. If the values during the analysis period were continuously +1.92 SD ($p < 0.01$) greater than the mean during the pre-Go period for 40 ms, then the neuronal activity was regarded as movement-related. For the 16 raster and histogram displays, the most strongly movement-related condition was determined using the following two criteria: (1) the neuronal onset was

shortest from the Go signal onset (neuronal reaction time), and (2) the peak frequency rate was higher than the others with similar neuronal reaction times under multiple conditions. Importantly, if neurons responded to the visual stimulus for conditional and spatial instruction signals (Figure 1), this was labeled signal-related activity (Weinrich and Wise 1982), and was excluded from analysis, even when the above two criteria were fulfilled.

Next, using the neuronal raster displays aligned at movement onset, the spike bursts of the movement-related activity in each raster that covered 1 s before and after eye or hand movement onset (see red dots in raster displays of Fig. 5) were detected based on the statistical differences in interspike intervals according to the Poisson distribution (Hanes et al. 1995); the Matlab code is open to the public and available at http://www.psy.vanderbilt.edu/faculty/schall/scientific-tools/. If multiple spike bursts were detected in a single raster, one of the bursts closest to the movement onset was selected for analysis. The first and last spikes of the bursts were defined as neuronal activity onset and offset, respectively. Additionally, the mean discharge rate of each spike burst was obtained. Using the burst data, the modulation of neuronal activity related to the same hand or eye movements between the Both and Hand or Eye trials was quantitatively examined if the activity was similar or differed depending on trial type. The modulation index was calculated using the following equation:

$$Modulation\ Index = \frac{discharge_{Both} - discharge_{Hand\ or\ Eye}}{discharge_{Hand\ or\ Eye}},$$

where $discharge_{Both}$ and $discharge_{Hand\ or\ Eye}$ refer to the mean discharge rate of the burst in the Both and in the Hand or Eye task, respectively. If the activity was the same in the two tasks, the index was zero. On the other hand, positive and negative index values indicated either an increase or decrease, respectively, in frequency rate in the Both task compared to the Hand or Eye task.

Additionally, an index of directional preference was calculated for each neuronal activity using the onset, offset, and mean discharge rate of the burst under the most movement-related condition; the direction of the target for which the neuronal activity exhibited the highest modulation was defined as the preferred direction. Next, the mean discharge rate under the condition with movement direction opposite that in the most-related condition during the period between the burst onset and offset under the most-related condition were obtained because the neurons usually exhibited different temporal discharge patterns for movement in the opposite direction. The direction index was calculated using the following equation:

$$Direction\ Index = \frac{discharge_{pref} - discharge_{opposite}}{discharge_{pref} + discharge_{opposite}},$$

where $discharge_{pref}$ and $discharge_{opposite}$ refer to the mean discharge rates during sampling periods with movement in the preferred direction and toward the direction opposite the preferred direction, respectively. If a neuron exhibited an activity change in only one direction and no activity in the opposite direction, the

direction index was 1.0. On the other hand, the index was 0.0 if the activity change was identical in the two directions. Additionally, EMG activity was similarly analyzed using the criteria for the neuronal analyses.

Histological reconstruction of recording sites

At the completion of the experiment, electrolytic marking lesions were produced by passing 20 μA of cathodal direct current through the microelectrodes for 15 s. Then, 9–10 days later, the monkeys were deeply anesthetized with pentobarbital sodium (50 mg/kg, i.m.) after induction of anesthesia with ketamine hydrochloride (8 mg/kg, i.m.). Monkeys were perfused through the heart with saline, followed by a fixative containing 3.7% formaldehyde in 0.1 M phosphate buffer (pH 7.4) and then 10% and 20% sucrose solutions in 0.1 M phosphate buffer (pH 7.4).

After marking the location of the recording chamber with five pins at known electrode coordinates, each brain was removed from the skull and photographed. Subsequently, the brain was serially sectioned (50 μm slices) in a horizontal plane with a freezing microtome, and images of the brain block were taken immediately before the individual sections were obtained using a digital camera (IXY Digital 600, Canon; Tokyo, Japan) placed above the microtome. The sections were stained with thionin, and the images were digitized with a scanner (GT-F600, Epson; Suwa, Japan). A three-dimensional reconstruction of the cortical volume was constructed using the digital images, and then the recording sites were matched with the volume using the electrode tracks and electrolytic marking

lesions; re-sliced images parallel to the electrode tracks were obtained. Finally, flattened reconstructions of the periarcuate cortex along the arcuate sulcus were produced by straightening layer V (Dum and Strick 1991; Gregoriou et al. 2005), and the exact locations of the recorded neurons were identified on the flattened images. A program was developed using Matlab 2015b to perform the histological reconstructions (Saga et al. 2011). The fundi of the arcuate sulcus and arcuate spur were used to delineate the FEF, PMv, and PMd (Gerbella et al. 2007; Matelli et al. 1985; Petrides et al. 2005).

RESULTS

Behavioral and EMG analyses

The behavioral analyses confirmed that, in successfully rewarded trials, the monkeys executed the reaching movements using only the required effectors, and also maintained their position within the required zones for 200–500 ms until a reward was obtained. Figure 2 illustrates hand and eye movement trajectories in the three tasks. In the Hand task, the hand positions hit the center of each target zone, while the eyes were fixed in the central holding zone. Conversely, in the Eye task, the saccades hit the center of the target zones, while the hand position was held in the central holding zone. In the Both task, the trajectories of the eye and hand movements were similar to those in the Eye and Hand trials, respectively. These findings were confirmed by comparing the velocity profiles among the three tasks; they were almost identical (data not shown).

Movement onset and target acquisition were detected based on each movement trajectory, and RT and MT values were defined as the time from the Go signal to movement onset and the time from movement onset to target acquisition, respectively. Table 1 shows the RT and MT values of the eye and hand movements in each task throughout the recording periods. In both monkeys, the mean RTs and MTs of the saccades were shorter than those of the hand movements. Because it was crucial to know if the eye and hand movements were coordinated in the Both task, the relationship between the eye and hand RTs was examined; Figure 3 shows the scatter plots of the RTs for the two monkeys. The two RTs of Monkey 1 were highly correlated (correlation coefficient $r^2 = 0.83$),

whereas those for Monkey 2 were not (r^2 = 0.17). The slope values of the least-squares lines for the data from Monkeys 1 and 2 were 1.05 and 0.06, respectively (sold line for Monkey 1 in left panel, dashed line for Monkey 2 in right panels of Fig. 3). However, when a two-dimensional Gaussian function was fit to the data, the major cluster represented by the ellipses in Figure 3 exhibited a much longer longitudinal axis relative to the minor axis. The slope of the least-squares line for the data from Monkey 2 around the larger eclipse was 5.01 (solid line in right panel). This implies that both Monkeys 1 and 2 performed coordinated eye and hand movements in a majority of trials.

The present study also analyzed EMG activities that were bilaterally recorded from various muscles (see Materials and Methods). The right triceps brachii muscle was found to be a prime mover, and the triceps brachii muscle (Fig. 4) and other upper arm muscles were similarly active in the Hand and Both tasks; no or only slight activities were observed in these muscles during the Eye task. Moreover, muscles including the right triceps brachii (Fig. 4) did not show changes in activity during the preparation periods prior to the presentation of the Go signal.

Discharge properties of movement-related neurons based on task mode

Neuronal activities in the periarcuate cortex and M1 of the left hemisphere were recorded during task performance. We recorded 982 task-related neurons in the cortical areas (540 and 442 neurons in Monkeys 1 and 2, respectively). As shown in previous studies (Bruce and Goldberg 1985; Kakei et al. 2001; Kalaska

and Crammond 1992; Kurata 1993; Kurata and Hoshi 2002; Kurata and Tanji 1986; Rizzolatti et al. 1981; Schall 1991a; Weinrich and Wise 1982), task-related activities included (1) activity change during a holding period before presentation of visual instruction signals (anticipatory activity); (2) phasic activity responding to visual signals for instructions (signal-related activity); (3) sustained activity during the instructed preparation periods (preparation- or set-related activity); (4) activity change during the interval between onset of the Go signal to initiate reaching and the end of the reaching trial, signaled by the delivery of a reward (tentatively, movement-related activity; see strict definition below); and (5) activity during inter-trial intervals. Among theses task-related activities, we focused on the tentatively termed movement-related activity in this study (see Introduction). The other types of task-related activities, e.g. preparation-related activity, will be described in a separate report. We defined the tentatively defined movement-related neurons using the following criteria. First, neurons with brief short-latency activity in response to visual instruction signals were excluded because the response was indistinguishable from movement-related activity immediately after the Go-signal. Next, the activity was stably recorded during at least 10 trials each among the 12 trial types (see Material and Methods), allowing subsequent quantitative analyses. Finally, burst activity during the interval was detected using differences in the Poisson distribution (see Materials and Methods). The activity of 374 neurons (187 neurons from each monkey) fulfilled these criteria; these will be described hereafter.

Several types of movement-related neuronal activity were classified in the present study. First, changes in neuronal activity that were closely associated with hand movements were observed. These neurons were active in the Hand and Both, but not in the Eye, trials, similar to the right arm EMG activity (Fig. 5A); these are termed Hand neurons. Second, a subset of neurons were active only when saccades were executed in the Eye and Both, but not Hand, trials (Fig. 5B); these are termed Eye neurons and were most frequently recorded in the FEF (Bruce and Goldberg 1985; Kurata 1993; Kurata and Hoshi 2002). A third category included a subset of neurons that were active regardless of task (Hand, Eye, and Both tasks; Fig. 6C); these are termed All neurons. The All neurons were recorded in the PMv at the depth of the arcuate sulcus, and became active approximately at movement onset and sustained their activity until reward delivery; this type of change was not observed in the EMG data of any recorded muscles (Fig. 4) but was frequently observed in other All neurons. Although the neuron shown in Figure 5C exhibited activity changes during the three tasks, its discharge pattern differed slightly among the tasks. First, the length of the bursts was constant during the Eye task, unlike during the Hand and Both tasks. This feature could be attributed to the temporally and metrically more stereotypic nature of saccadic eye movements than those of the hand movements performed in the Hand and Both tasks. Second, discharge frequency was higher during the Both task than during the Hand task, even though hand movements were similar in the two tasks (Fig. 2). This observation could imply that the neuronal activity was more modulated during coordinated eye-hand movements than in hand movements without accompanying

eye movements. The latter feature was quantitatively analyzed and will be described in following sections. In addition to these three types of movement-related activity, activities specifically related to either the Both, Hand, or Eye tasks were also observed (Fig. 5D–F); these are termed Both Only, Hand Only, and Eye Only neurons, respectively. It should be noted that the Eye Only neuron shown in Figure 6F changed its activity after saccade onset in the Eye trials, but this change was not considered a visual response during visuospatial target acquisition because the eye movements were identical in the Eye and Both trials. Lastly, only one neuron recorded in the PMv exhibited activity in both the Hand and Eye tasks, but not in the Both task (data not shown); this neuron was termed a Hand and Eye neuron.

Locations of movement-related neurons in the periarcuate cortex

Figures 6 and 7 show the locations and numbers, respectively, of the classified neurons in the investigated subregions (M1, PMd, PMv, and FEF). To identify the recording sites in neurons within the arcuate cortex, three-dimensional histological sections were reconstructed and then re-sliced to obtain sections parallel to the electrode tracks (see Materials and Methods). The region of the periarcuate cortex, which is near where the arcuate spur merges with the arcuate sulcus was of particular interest in the present study and neurons from the surface of this region to the fundus of the sulcus were recorded. The examined areas contained the PMd near the precentral dimple, from which task-related neurons have been frequently recorded (Cisek and Kalaska 2004; Cisek and Kalaska 2005;

447 Hoshi and Tanji 2002; 2000; Kurata 1993; Pesaran et al. 2006). In the present
448 study, the pre-arcuate cortex was regarded as the FEF, although a small part of the
449 prefrontal cortex rostral to area 8 was also included (Fig. 7).

450 Hand neurons were primarily located in M1 (40 of 51 neurons, 78%), the
451 PMd (38 of 77 neurons, 49%), and the PMv (50 of 140 neurons, 36%) caudal to
452 the arcuate sulcus (right panels, Fig. 6). Eye neurons were primarily located in the
453 FEF (70 of 111 neurons, 63%), PMv (21 of 140 neurons, 15%), and PMd (10 of
454 140 neurons, 13%) within the arcuate sulcus. In contrast, All neurons were
455 recorded in all explored regions but most frequently identified in the PMv (36 of
456 140 neurons, 26%) in the posterior bank of the arcuate sulcus. The percentage of
457 All neurons was much higher in the PMv relative the other areas (14 of 141 FEF
458 neurons, 10%; 11 of 77 PMd neurons, 14%; and 4 of 51 M1 neurons, 8%). Other
459 types of neuronal activity were scattered throughout the periarcuate cortex, but the
460 activity trends were similar in both monkeys.

461

462 *Temporal relationship of neuronal bursts to movement onset and reward delivery*

463 The present study also aimed to determine whether the burst activity of
464 periarcuate neurons would exhibit different temporal profiles relative to the
465 various events associated with reaching movements from the time the Go signal
466 was presented until successful reaching was signaled by reward delivery. To
467 characterize the temporal profiles of movement-related activity, a spike burst from
468 each raster from 1 s before and after movement onset was extracted by detecting
469 differences in the Poisson distribution of the spike train (see Materials and

Methods); when multiple spike bursts were detected, the spike burst nearest movement onset was selected. For data recorded in the Both task, a burst in a raster aligned with the hand and eye movement onsets was extracted. Next, the most related of the 16 trial types (four directions among the Eye, Hand, and two Both task conditions aligned at eye and hand movement onsets) in which a spike burst with the highest discharge rate was present was selected. For neurons that were most related in the Both task, the neuronal onset time with a smaller SD from the hand or eye movement onsets was selected.

Figure 8 illustrates the neuronal bursts of the Hand, Eye, and All neurons aligned with the hand or eye movement onset. Several general trends were identified throughout the cortical areas: (1) the bursts in each area exhibited various onsets during the period between presentation of the Go signal and reward delivery (marked by gray dots in Fig. 9), and (2) the burst lengths of most neurons were not long; some neuron bursts terminated prior to movement onset, whereas a small number of neurons exhibited relatively long-lasting activity that was sustained until approximately the time of the reward delivery.

To further characterize neuronal profiles in the cortical subregions, three major timing variables were examined: (1) whether the neuronal onset preceded or lagged behind the movement onset; (2) whether there were any differences in the duration of movement-related activity (from neuronal onset to offset; see Experimental Procedures); and (3) how the activity was associated with the reward delivery that signaled success of a trial. These three aspects were analyzed by creating cumulative-sum histograms of the timing (Fig. 9). The neuronal burst

493 onset varied from -500 to 500 ms around the movement onset, and the duration
494 varied from 100 to 300 ms. There were no significant differences between
495 neuronal classifications or between cortical areas in terms of burst onset or
496 duration (analysis of variance [ANOVA], $p > 0.05$), except that the burst onset of
497 the Both neurons in the PMv, PMd, and M1, and their offsets in the PMd appeared
498 significantly later than those in the other classifications (ANOVA, $p < 0.05$).

499 The present study also analyzed the burst offsets relative to the reward
500 delivery (Fig. 10) and found no statistically significant differences among
501 neuronal classifications (ANOVA, $p > 0.05$). However, the All neurons frequently
502 exhibited sustained activity until reward delivery (Fig. 5). Thus, a sampling period
503 of 150–0 ms before reward delivery (shaded area in the All panel, Fig. 9B) was
504 selected and the percentage of neurons whose bursts ended during this period was
505 calculated. The results showed that the activity of 63.6% (7 of 11) of All neurons
506 terminated during this period (marked by a red arrow in the PMd Offset panel of
507 Fig. 9A), whereas the spike bursts of only 36.1% (13 of 36) and 28.6% (4 of 14)
508 of All neurons in the PMv and FEF, respectively, ended within this timeframe.
509 The overall percentage of All neurons whose activity was terminated during this
510 period was 40.0%. Of the Hand neurons, the bursts of 34.0% (17 of 50), 28.9%
511 (nine of 38), and 15.0% (six of 40) of neurons in the PMv, PMd, and M1,
512 respectively, terminated during this period. Of the Eye neurons, bursts of 30.0%
513 (21 of 70), 19.0% (four of 21), and 10.0% (one of 10) of the neurons in the FEF,
514 PMv, and PMd, respectively, ended during the period. Thus, the proportion of
515 neurons whose bursts terminated during the period close to the reward delivery

was higher for the All neurons in the PMv and PMd than for the Hand and Eye neurons in these regions (Pearson's Chi-square [χ^2] test, $p < 0.05$).

Directional profiles of neuronal activities in periarcuate neurons

The directional preferences of the classified neurons in the present study were also analyzed (Fig. 10, Table 2). A majority (235 of 305 neurons, 77%) of the Hand, Eye, and All neurons exhibited significant differences between their preferred and opposite directions (two-tailed t-test, $p < 0.05$, darkly hatched histograms in Fig. 14); the preferred directions were almost evenly distributed (Table 2). Although more accurate preferred directions could have been calculated if we had trained the monkeys to perform a task with eight directions (Georgopoulos et al. 1982), instead of four directions, our data approximated the values. All of the classified neurons in the PMd (100%) showed a significant directional preference (two-tailed t-test, $p < 0.05$), and similar trends were found in the other areas. However, the populations of neurons that did not show significant directional preferences (lightly shaded histograms in Fig. 10) were higher in the PMv (46% of Hand neurons [23 of 50], 43% of Eye neurons [nine of 21], and 39% of All neurons [14 of 36]) than in the PMd and FEF (Pearson's χ^2 test, $p < 0.05$).

Modulation of activity based on task mode

When neurons, such as Hand neurons, were active in both the Both and Hand tasks, their movement-related discharge rates were not always constant. For

example, the All neuron shown in Figure 6C was more active in the Both than in the Hand task. Figure 11 presents histograms of the modulation indices (see Materials and Methods) for neurons classified as Hand, Eye, and All; several of the classified neurons exhibited modulated increases (Modulation Index >0) or decreases (Modulation Index <0). In all, 40.7% of the neurons (124 of 305 examined neurons) exhibited statistically significant modulations; of these, 60.0% (30 of 50) and 44.7% (17 of 38) of the PMv and PMd Hand neurons, respectively, and 52.8% (19 of 36) of the PMv All neurons predominated. Alternatively, these data indicate that 59.3% of the neurons did not exhibit any type of modulation. More specifically, 67.1% (47 of 70), 61.9% (13 of 21), and 100.0% (10 of 10) of Eye neurons in the FEF, PMv and PMd, respectively, showed no significant modulation, and 65.0% (26 of 40) of Hand neurons in M1 showed no significant modulation.

No modulation of activity by various eye and hand reaction times

During the Both task, the monkeys were required to make coordinated eye-hand movements. It can be assumed that when hand and eye RTs were closer, the movements were more coordinated. It is then possible that neuronal activity is higher when hand and eye RTs were near the correlation line shown in Figure 3 than when they were distant from the line. We calculated distances from the correlation line to the hand and eye RT data points during Both trials. The distance indicates that when hand RT was longer, the eye RT was inversely shorter within a trial than in correlated trials, and vice versa. The distance values

above and below the least-squares line were signed positive and negative, respectively. We obtained the mean discharge rate of the neuronal burst in the best direction of the Both task. The discharge rate in each trial was then divided by the mean frequency among all trials to obtain the normalized value. Similarly, the distance of a hand and eye RT data point obtained from the least-squares line was divided by the maximal distance of the whole population for each monkey shown in Figure 3 to obtain a normalized distance value. For each neuron, a regression line for the normalized data on a scatter plot was created, and its slope value was obtained. If the slope was negative, then the neuron discharged more vigorously when hand and eye RTs were closer to the regression line shown in Figure 3. Representative data are shown in Figure 12A. The activity was recoded in the PMv and was classified as that of a Hand neuron. The least-squares line of the scatter plot had a slope of –0.28; there was no statistically significant correlation between the distance and the activityt ($p > 0.05$; correlation coefficient determined using Matlab). Thus, the neuron did not modulate its activity depending on the distance. Figure 12B shows cumulative-sum histograms of the slope values of Hand, Eye, All, and Both Only neurons in the four cortical areas. A majority of the slopes approached zero, indicating a trend. Although 11 of 172 neurons in Monkey 1 (6.3%) and 11 of 178 neurons in Monkey 2 (6.2%) exhibited statistically significant modulation ($p < 0.05$; correlation coefficient determined using Matlab), their slope values were nearly zero (0.418 ± 0.20 (mean and standard deviation) for six positive values (three data points per monkey), and -0.29 ± 0.24 for 16 negative values (eight data points per each monkey)). Thus, no

585 classified neurons exhibited modulation, even when hand and eye RTs were on
586 the correlation line. The trends were similar in two monkeys shown in Figure 12.
587 We also analyzed the data using positive and negative values of distance instead
588 of absolute values as above; however, the results were similar: most neurons did
589 not exhibit modulation depending on distance from the correlation lines. These
590 results show that, in both monkeys, better correlated hand and eye RTs did not
591 more effectively activate the neurons.

592 Using the same methods, we also examined the relationship between the
593 executed movement metrics and dynamics and the absolute distance from the
594 correlation line shown in Figure 3. We chose hand and eye movement amplitude
595 for motor metrics, and maximal hand and eye velocities as motor dynamics. We
596 analyzed data collected during the 350 sessions shown in Figure 12, and present
597 the results in Figure 13. Because the monkeys made accurate hand-eye
598 movements to the targets and the maximal eye velocities were nearly identical in
599 every trial, the data on the curve were concentrated near the zero value. The
600 general trends were similar in two monkeys (Fig. 13). Among the movement
601 metrics and dynamics, maximal hand velocity data showed more modulation than
602 did other parameters (Fig. 13). However, only 4.7 and 2.8% of the data for
603 Monkeys 1 and 2, respectively, exhibited statistically significant modulation ($p <$
604 0.05; correlation coefficient determined using Matlab).

DISCUSSION

The main finding of the present study was that neurons in the periarcuate cortex exhibited a wide range of movement-related activity patterns when coordinated eye-hand reaching movements were executed. These patterns were classified into three major types: (1) those dependent on a specific effector (eyes or a hand); (2) those independent of the effectors, termed All-related activities; and (3) those specific to task type. The neurons were active during a period that encompassed the initiation, execution, and completion, as signaled by the delivery of a reward, of the reaching movement. Additionally, the neuronal activities were more frequently modulated during coordinated movement in the Both task than in the Hand and Eye tasks. The functional roles of the classified neurons in the subregions of the periarcuate cortex during a reaching movement involving hand-eye coordination will be discussed based on their particular characteristics and locations.

Functional subdivisions in the periarcuate cortex

Consistent with previous findings (Bruce and Goldberg 1985; Kiani et al. 2015), the present study showed that a majority of FEF neurons in the pre-arcuate cortex were active in association with saccadic eye movements; these neurons were termed Eye neurons. In the present study, the Eye neurons exhibited changes in contraversive (33%) as well as ipsiversive activities (20%), which is consistent with the results by Schall (1991), although other studies reported that most FEF saccade-related neurons were contraversive (Bruce and Goldberg 1985; Tanaka

628 and Fukushima 1998). This discrepancy may be due to the fact that the regions
629 investigated in the present study covered a wider area, including a caudal portion
630 of the prefrontal cortex and the deep part of the rostral bank of the arcuate cortex,
631 compared with previous studies.

632 In the postarcuate cortex, activity in neurons that closely related to hand
633 movements, termed Hand neurons, was frequently recorded in M1 and in the
634 PMD and PMv, as previously reported (Kurata 2007; Kurata and Hoshi 2002).
635 Thus, the PMd and PMv can be regarded as regions that control limb movements.
636 However, the present study also showed that the postarcuate cortex, consisting of
637 the PMv and PMd, contained neurons related to saccades (Tanaka and Fukushima
638 1998). This region was initially thought to control smooth-pursuit eye movements
639 (MacAvoy et al. 1991; Tanaka and Fukushima 1998; Tanaka and Lisberger 2002),
640 and stimulation of the PMv evokes saccades (Fujii et al. 1998).

641 The neuronal signals closely linked to the effectors, designated as Hand and
642 Eye neurons, seemed to convey motor commands from the periarcuate cortex
643 during eye and hand movements. Although the descending projections of these
644 neurons were not labeled in this study, motor commands that convey hand
645 movements can be transmitted to the spinal cord either directly (Dum and Strick
646 1991; Lemon 2008; Shimazu et al. 2004) or indirectly via M1 (Muakkassa and
647 Strick 1979; Rubino et al. 2006). On the other hand, commands for saccades could
648 be sent to the superior colliculus (Segraves and Goldberg 1987) or the dorsal
649 pontine nuclei (Tziridis et al. 2009). However, the present study demonstrated that
650 the differences between the pre- and postarcuate cortices were not absolute

651 because two types of classified neurons that were closely linked to the effectors
652 were distributed throughout the three studied brain regions; thus, Hand neurons
653 were more frequently recorded in the PMd and PMv than in the FEF, whereas Eye
654 neurons were recorded in the FEF more frequently than in the PMd and PMv.
655 Accordingly, it may be more appropriate to state that functional gradients
656 organized in a rostrocaudal manner from FEF to M1 exist and that each of these
657 commands should be organized to coordinate reaching movements involving the
658 eye and the hand.

659 Although many of the classified neurons exhibited an affinity for the
660 effectors, task-specific and task-nonspecific activities that were not similar to the
661 effector activities were identified as well. The periarcuate cortex, consisting of the
662 FEF, PMv, and PMd, contained All neurons that were similarly active during the
663 three tasks regardless of the effector that was signaled. Neurons of this type were
664 recorded throughout the periarcuate cortex, but they exhibited the densest
665 distribution in the PMv. The role of the All neurons in the coordinated eye–hand
666 movements will be discussed in the following section.

667 In addition to the three major categories (Hand, Eye, and All), the present
668 study also identified neurons that exhibited task-dependent selective activation.
669 These task-specific neurons were termed Both Only, Hand Only, and Eye Only
670 neurons (Figure 6); they may represent parcellated or categorized commands that
671 can be flexibly integrated into the final motor commands based on behavioral
672 demands; this type of eye–hand activity has been previously reported in the PMv
673 (Tanji et al. 1987), pre-supplementary motor area (Shima and Tanji 2000), FEF,

and supplementary eye field (Mushiake et al. 1996). Much like the three main categories of neurons, these neurons may play a role in executing specific reaching movements using the eyes and/or a hand, or in monitoring one's own behavior in a task-specific manner, as many of these neurons were activated after the onset of reaching. The fact that such a wide variety of neurons is involved in eye–hand reaching movements may imply that the periarcuate cortex, as a whole, contributes to the coordination of such movements by orchestrating the activities of these neurons.

Temporal cascade of changes in neuronal activity from the initiation to the completion of a reaching movement

In the present study, the neurons exhibited different discharge onsets and patterns depending on direction and task mode. Thus, neuronal bursts with discrete onsets and offsets were extracted from the spike population to characterize their activity using Poisson spike train analyses (Hanes et al. 1995). Using a statistical definition to identify neuronal bursts in each raster, it became clear that the duration of the bursts tended to be shorter than when they were analyzed using conventional histograms or spike density analyses and that the absence of a spike burst did not necessarily mean there was no activity in the raster. Thus, the neuronal activity was extracted only when it exhibited a statistically significant increase above baseline activity.

The MTs for hand reaching were much longer than those for saccades, and the number of muscles involved in the forearm engaged in reaching was much

greater than that of neurons involved in saccades (maximally six extraocular muscles per eye). During hand reaching, the activity patterns of various muscles are spatially and temporally different in relation to the initiation, execution, and termination of the synergistic movements (Berniker et al. 2009). Due to the temporal and spatial complexity of multi-joint movements, the neuronal activity that started changing at various times in the present study may reflect the sequential commands necessary for multi-joint movements (Fig. 10, Hand). This type of limb movement may be more complex than saccadic eye movements, which are controlled by six extraocular muscles directly attached to the eyeball (Becker 1989). Regarding the Eye neurons in the present study, only half of the neuronal population related to saccades exhibited activity preceding the saccade onset, and the other half changed activity after the saccade (Fig. 10). This pattern held true for all classified neurons that showed a change in activity that preceded and lagged behind movement onsets (Fig. 10).

In the present study, the monkeys were required to fixate on a peripheral target in both the Eye and Both tasks, as well as acquire the target with a hand while fixating on the central holding zone with their eyes. It was expected that the monkeys would monitor their eye and hand positions and anticipate the successful completion of the reaching by awaiting reward delivery. When the offsets of the neuronal bursts in relation to reward delivery were analyzed, the Hand neurons in the PMv and FEF and the All neurons in the four studied areas were active after the completion of the movement until approximately the time of the reward delivery (Figs. 9 and 10). This suggests that the studied cortical regions are

720 involved in the cognitive processes that ascertain the completion of reaching
721 behavior.
722
723 *Modulation of activities during performance of coordinated behaviors*
724 In the present study, a majority of the periarcuate neurons exhibited
725 modulated activity when coordinated movements were executed relative to the
726 neurons that were active during hand or eye movement (Fig. 11). The modulations
727 included both increases and decreases in spike discharge rates. An increase in
728 activity during the Both task may not be simply regarded as a result of increased
729 attention because the RT and MT were similar between tasks (Table 1). It is
730 possible that these modulations reflected dynamic processes between the
731 behavioral requirements. Some neurons showed an increase in discharge rates
732 during the Hand task relative to that during coordinated movements. This can be
733 interpreted to mean that, because humans typically perform movements that
734 require the coordination of eye and hand activities, precise reaching behaviors
735 without eye movements would be unusual, and require more energy to complete.
736 In support of this notion, it was relatively difficult to train the monkeys in the
737 present study to perform hand movements without eye saccades in the Eye task.
738 We also examined whether movement-related neurons were modulated when
739 hand and eye RTs were highly correlated. However, a vast majority of the neurons
740 did not show such modulation. No relationship between movement metrics and
741 the RTs was observed. Combined with our behavioral results showing that
742 executed eye and hand movements were stereotypic and not variable among trials,

743 these findings indicate that burst activities might be more closely linked to
744 constantly executed movements than to reaction times. Furthermore, the trial-to-
745 trial variability of burst frequencies (Fig. 12A) could be regarded as intrinsic noise
746 which is thought useful in generating motor commands for accurate and
747 successful movements (Harris and Wolpert 1998; Scott 2002; Todorov and Jordan
748 2002).
749
750 *Characteristics of the task non-specific versus task-specific activities*
751 A major finding of the present study was that All neurons showed changes
752 in activity in association with both eye and hand movements and that this
753 population was most frequently recorded in the PMv (Figs. 7 and 8). This
754 particular characteristic makes the PMv unique within the subregions of the
755 periarcuate cortex. Additionally, these neurons exhibited less directional
756 preference than did the neurons involved in other activities, including Hand and
757 Eye neurons (Fig. 12). Thus, it is possible that the All neurons whose activity
758 onsets preceded the movement onset could be a universal prototype underlying a
759 command for reaching that involves the eyes and a hand and that this activity is
760 serially transformed to motor commands for use by specific effectors. If so, the
761 onset of All-related activity may emerge earlier than that of effector-dependent
762 activities, such as Eye or Hand activities. However, this idea is not fully supported
763 because the neuronal onset times of the All neurons did not precede those of
764 specific effector-linked Hand and Eye neurons (Fig. 10).

765 Although the All neurons were universally active regardless of whether the
766 reaching was performed using the hand, eyes, or both, a number of these neurons,
767 in conjunction with other types of neurons, were active during maintenance of the
768 post-reaching position (Figs. 9 and 10). This property is of particular interest,
769 considering their role in coordinated hand–eye reaching movements, because the
770 neurons were only active during the reaching movement with the hand and not
771 during the fixation period prior to reaching. Thus, it is possible that neuronal
772 activity during the post-reaching period may reflect self-monitoring of ongoing
773 reaching behavior until that action is completed. Although this hypothesis should
774 be clarified in future studies, the present interpretation may correspond to
775 previous findings showing that corticospinal neurons in the PMv exhibit mirror
776 properties that monitor one's own actions as well as the actions of others and that
777 these neurons might play a role in the suppression of the action (Kraskov et al.
778 2009).

779 We must consider at least three alternative interpretations of this activity.
780 The first is that these neurons play a role in fixation. Neurons in the ventral FEF
781 surface are active during fixation (Izawa et al. 2009), which is consistent with the
782 present findings because the monkeys were required to hold (or not move) the
783 target positions of the eyes and hands during the post-reaching period. Moreover,
784 the FEF projects to the superior colliculus (Segraves and Goldberg 1987), and the
785 rostral pole of the superior colliculus plays a role in fixation (Munoz and Wurtz
786 1993). However, this interpretation may not be supported by the present
787 observations for two reasons. First, the neuronal activity observed in the present

788 study did not simply reflect eye fixation because the neurons were not active
789 during the instructed preparation period during which fixation was required.
790 Rather, they became active after the reaching movement regardless of the effector,
791 and exhibited sustained activity while the monkeys maintained their eyes and
792 hand in the required positions until the trial was completed. Second, the location
793 of the area did not necessarily correspond to the region where activity was
794 identified in the present study, namely primarily in the PMv posterior to the
795 arcuate sulcus and not in the ventral FEF as previously shown (Izawa et al. 2009).
796 The second alternative interpretation is that the activity may reflect a
797 holding posture during the interval between movement initiation and reward
798 delivery. We consider this possibility unlikely because the All neurons were not
799 active when subjects withheld their movements, and results were similar during
800 periods between trial initiation and the Go-signal. We recorded neuronal activity
801 during the preparation periods before movement. These activities will be
802 described in a separate report; however, their activity patterns were very different
803 from those of the All neurons; the All neurons did not necessarily exhibit
804 sustained activity change during the preparation period, as shown in Figure 5C.
805 The third interpretation is that the activity is a reflection of anticipatory
806 licking before fluid delivery. We think that this unlikely as well, for two reasons.
807 First, we recorded neuronal activity related to orofacial movements of licking, but
808 these were most frequently found in the other part of the PMv immediately lateral
809 to the region where the Hand neurons were observed in this study (Geyer et al.
810 2000; Kurata et al. 1985). Second, the activity of most of the orofacial neurons

was synchronized with multiple licking movements following reward delivery; however, the All neurons did not exhibit such an activity pattern immediately before and after reward delivery (Fig. 5C).

Conclusions

The present findings indicate that, as a whole, the various neuronal activities in the periarcuate cortex contribute to the initiation and execution of coordinated eye–hand movements as well as to monitoring of performance and confirmation of performing a successful behavior. The latter view is supported by previous findings showing that the PMv contributes to performance monitoring and decision making and to encoding the outcomes of a decision (Pardo-Vazquez et al. 2008; 2009). Thus, it may be proposed that the periarcuate cortex, which comprises a number of subregions, orchestrates coordinated eye–hand reaching movements, beginning with effector control and continuing until completion. In this manner, the periarcuate cortex may serve as a mission control center for reaching behaviors that require coordinated eye and hand movements.

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ACKNOWLEDGMENTS

We are grateful to Drs. E. Hoshi and K. Samejima who provided a program to obtain location of the recorded neurons in the flattened reconstruction image of the cerebral cortex.

GRANTS

This work was supported by Grant-in-Aid for Scientific Research on Priority Areas <System study on higher-order brain functions> (20020002) and for Scientific Research (C) (22500348) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT), and Grant for Hirosaki University Institutional Research. The Japanese monkeys were supplied by National BioResouce Project “Japanese monkeys” of the MEXT.

FIGURE LEGENDS

Figure 1. Behavioral task sequence. The monkeys initiated a trial by fixating their eyes and aligning a cursor (large cross) in the central zone. The effector(s) and reaching target were indicated separately during a delay period. Reaching using the hand, eyes, or both was indicated by a small red, green, or yellow square, respectively, in the central zone. The target was indicated by a small white square in the peripheral zone. The Go signal to initiate reaching was indicated by changing the small central square from white to blue. The monkeys were required to maintain the reaching position in the target zone for 200–500 ms to receive a juice reward.

Figure 2. (A) Behavioral data showing the trajectories of hand and eye movements during the Eye, Hand, and Both trials. The trajectories are plotted on the XY coordinates corresponding to the LCD display (1280 × 1024 pixels), where the central holding and target zones were displayed. (B) The horizontal and vertical trajectories of the hand and eye movements in the three tasks were aligned at the onset (vertical dotted lines) of the hand (Hand) and eye (Eyes and Both) movement tasks. Note that hand movements were initiated at varying times after saccade onset.

Figure 3. Scatter plots of the eye and hand reaction times (RTs) of the two monkeys during the Both task throughout the recording periods (n = 14876 for Monkey 1; n = 16874 for Monkey 2). The ellipses in each figure show the

contours of Gaussian distribution models one and two for Monkey 1 and Monkey 2, respectively. The regression lines (solid lines) are hand RT = 1.05 × eye RT – 25.5 and hand RT =5.01 × eye RT – 760.2 for Monkeys 1 and 2, respectively. The regression line for the entire Monkey 2 population (dashed line) is hand RT =0.06 × eye RT – 381.3.

Figure 4. Electromyographic (EMG) activity of the right triceps brachii aligned at the onset of hand movement in the Hand and Both trials and at saccade onset in the Eye trials.

Figure 5. Raster displays and spike rate histograms of six types (A–F) of reach-related neuronal activity (Hand, Eye, Both, All, Both Only, Hand Only, and Eye Only) aligned at the hand movement onset in the Hand and Both tasks (left and center columns, respectively) and at saccade onset in the Eye task (right column). For each neuron, the best movement direction (right, up, left, or down) was selected. Red dots in the raster displays indicate burst activity detected by statistical differences in the Poisson distribution of the interspike interval during the 2-s sampling period (see Materials and Methods for definition). The blue and cyan marks in each raster indicate Go signal onset and reward delivery, respectively. Hatched areas in the histograms show statistically significant increases in neuronal activity ($p < 0.01$) during the reach period (see Materials and Methods for details). Recorded areas of the neurons: PMv for A, C; PMd for D and F; and FEF for B and F.

Figure 6. Histogram showing the numbers of classified neuronal activities recorded in the cortical subregions (PMv, PMd, and FEF) of the periarcuate cortex and M1. Data from the two monkeys are combined.

.

Figure 7. Distributions of the classified movement-related neurons in the periarcuate cortex for the two monkeys. (A) Neurons exhibiting activities related to saccadic eye movements (Eye and Both trials). (B) Neurons exhibiting movement-related activities in trials that required hand movements (Hand and Both trials). The periarcuate cortex was flattened, the locations of the neurons were projected to layer V, and the distance from the fundus of the arcuate sulcus (the central dotted line) was measured. The gray dotted lines, one in the PM and another in the FEF, indicate convexities of the cortex facing the arcuate sulcus. (C) Reconstructed histological slices: colored dots indicate the entry points of electrodes penetrating to the four cortical regions (cyan, M1; blue, PMv; red, PMd; and green, FEF). Abbreviations: Cent, central sulcus; Arc, arcuate sulcus; Prin, principal sulcus.

Figure 8. Onsets, offsets, and durations of Hand, Eye, and All neuron bursts indicated by horizontal lines. They were sorted according to time in relation to movement onset (marked as 0 ms). Near the end of each line, the mean timing of reward delivery is indicated by a gray dot.

Figure 9. (A) Cumulative-sum histograms of the classified neurons' burst onset, duration, and offset, in the four cortical areas. The neuronal burst onset and offset were aligned at movement onset and reward delivery, respectively. As marked by a red arrow in the PMd-Offset panel, it is evident that a number of All neurons in the PMd terminated around reward delivery when their bursts were aligned at reward delivery. (B) The same offset data of the three major classifications of neurons (Hand, Eye, and All) shown in A rearranged at reward delivery. The lightly hatched area shows the period 150–0 ms prior to the reward delivery, when the bursts of a number of All neurons ended.

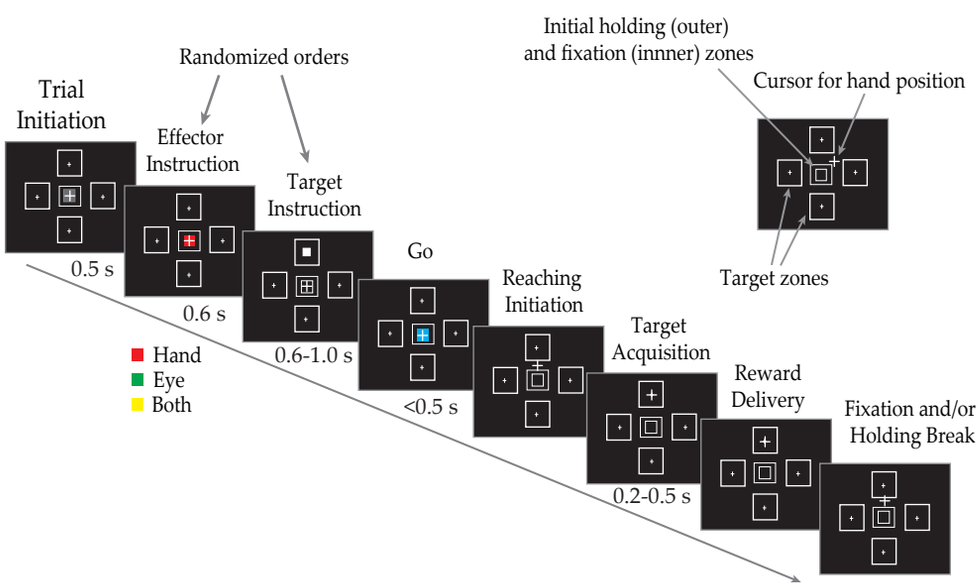
Figure 10. Direction indices calculated from the mean discharge rate in the most- and least-preferred movement directions under the same task mode, i.e., the Hand, Eye, or Both task (see Materials and Methods).

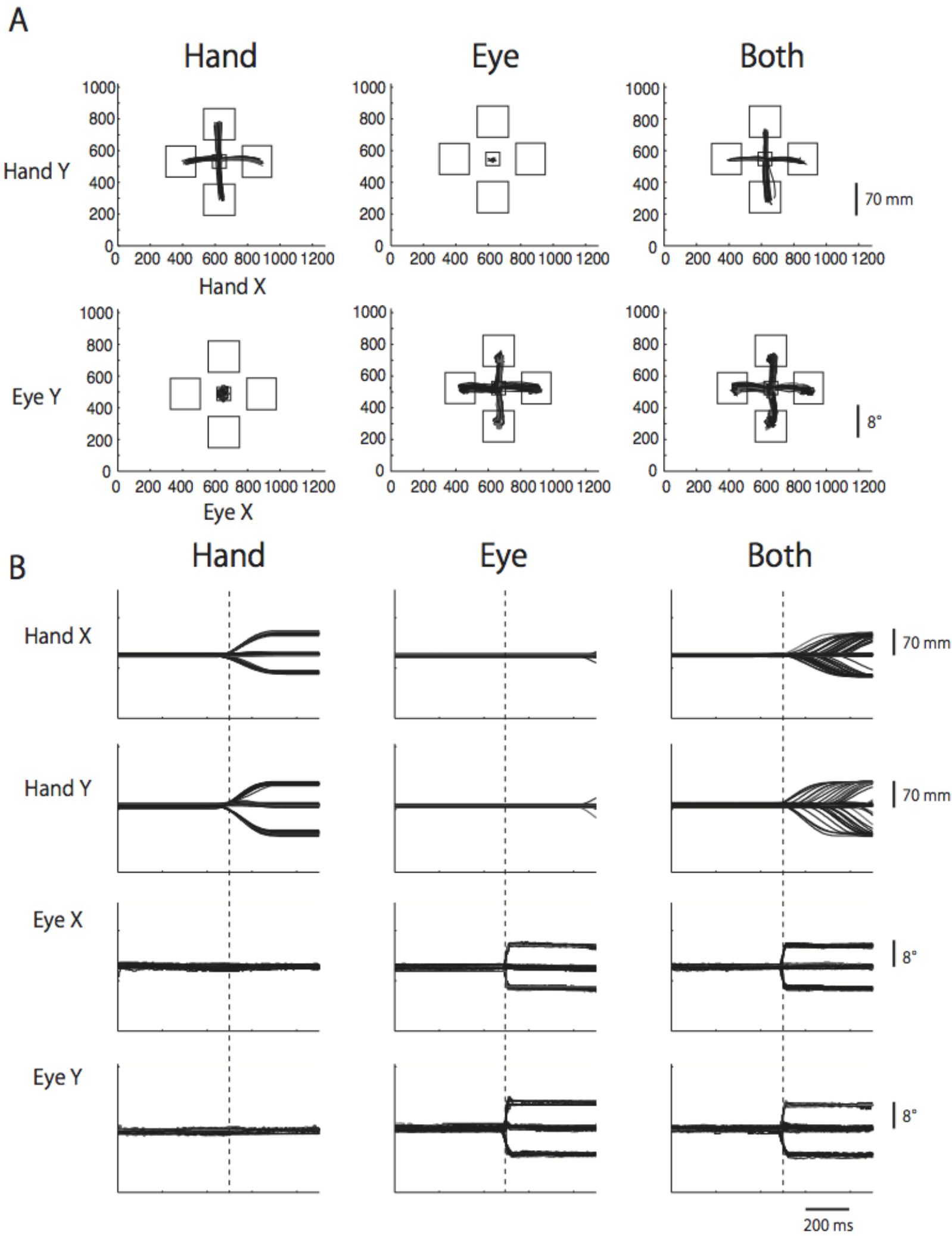
Figure 11. Modulation indices of the neuronal bursts of the Hand, Eye, and All neurons in the four cortical areas (see Materials and Methods). Neurons that showed statistically significant differences in frequency rate (two-tailed t-test, $p < 0.05$) are indicated by dark shades.

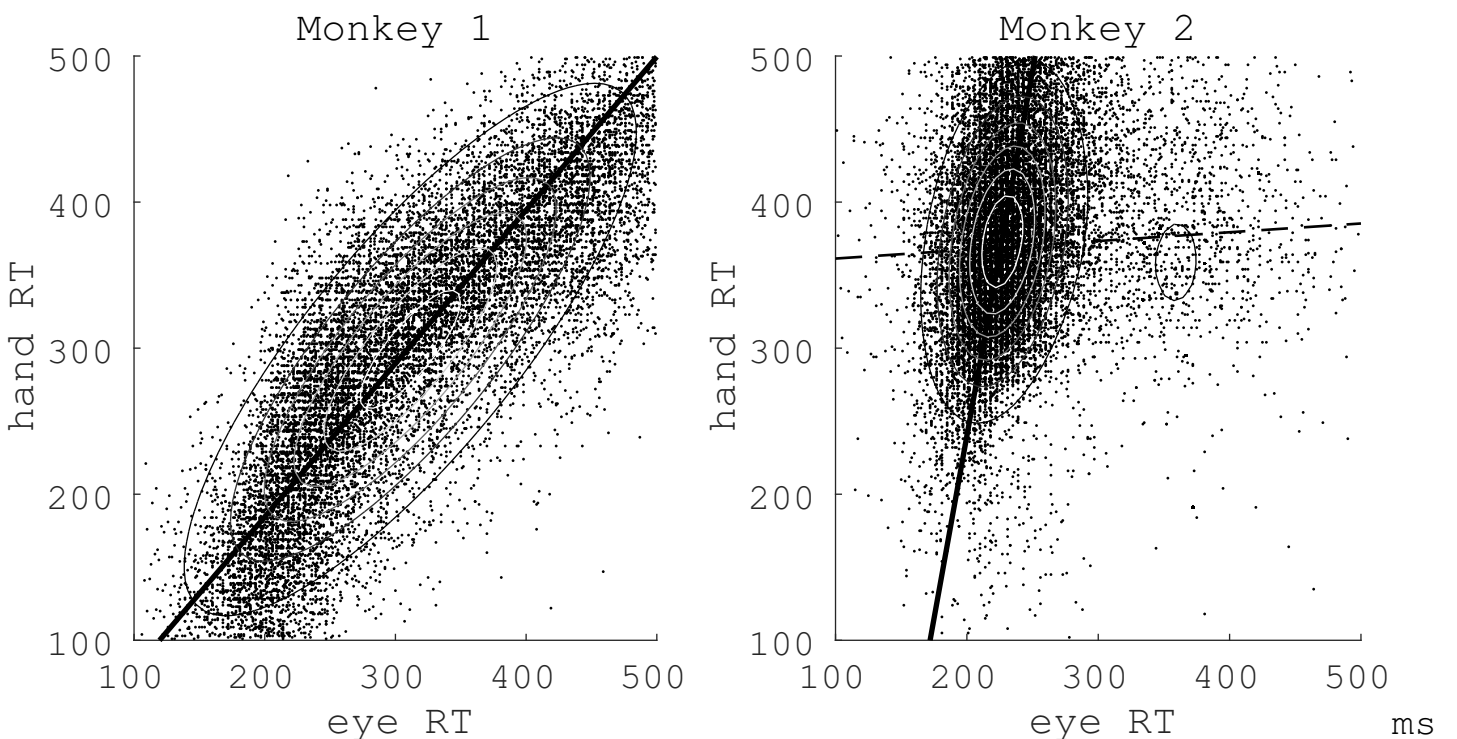
Figure 12. (A) Scatter plot of normalized activity modulation of a representative Hand neuron recorded in the PMv vs. normalized absolute distance from the correlation line between hand and eye RTs (Fig. 2). This analysis was performed to visualize whether neuronal activities were modulated depending on distance

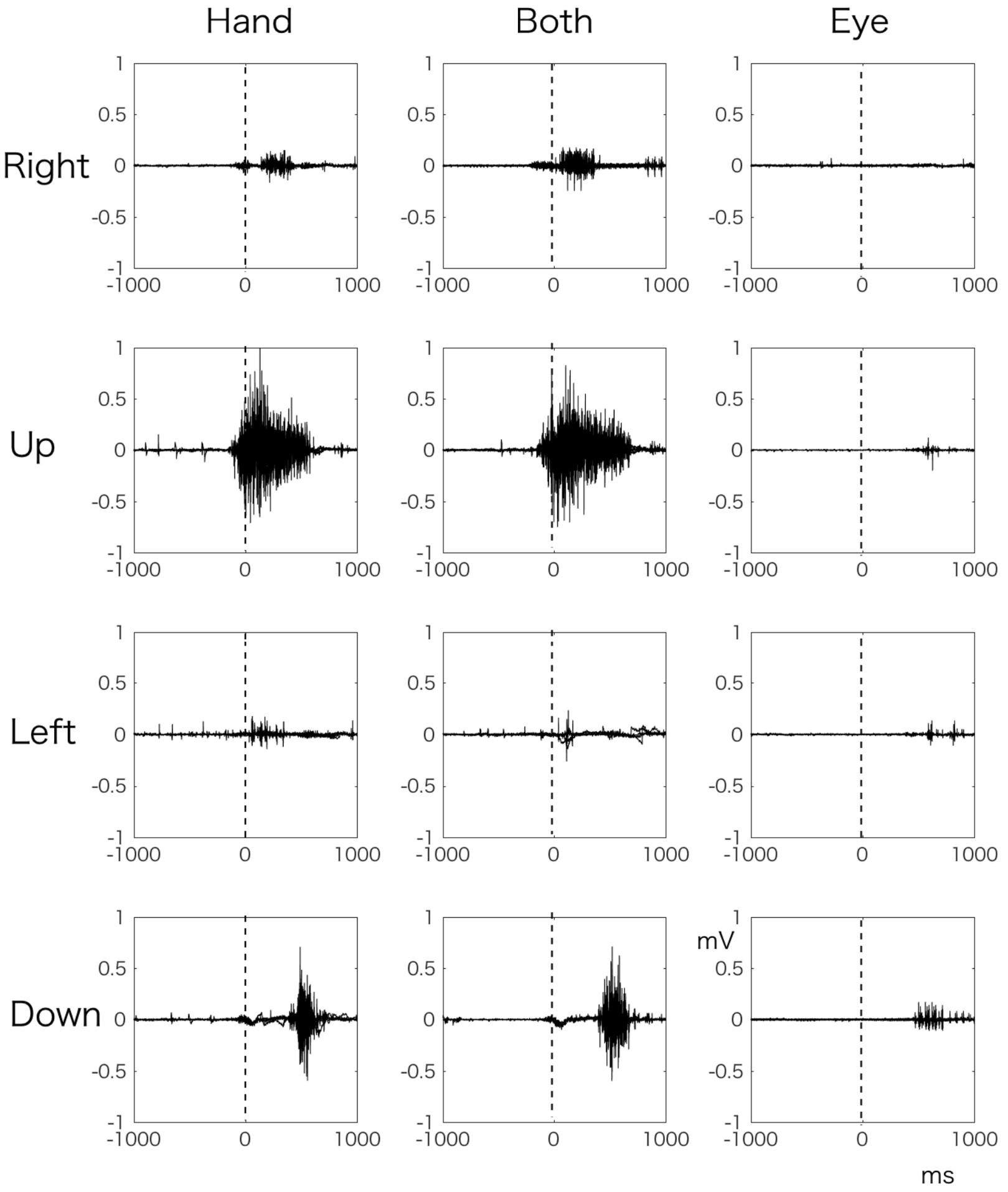
from the least-squares correlation lines shown in Figure 3. In the scatter plot, a least-squares line for the data is also indicated. The value of the slope was nearly zero (–0.28), and the correlation between the two values was not statistically significant ($p > 0.05$). (B) Cumulative sum histograms of the slopes of least-squares lines derived from scatter plots as shown in (A) for Hand, Eye, Both, and Both Only activities recorded in the four cortical areas during Both trials. See text for details.

Figure 13. Slopes of least square lines in scatter plots of normalized motor metrics (maximal amplitudes of hand and eye movements) and dynamics (maximal velocities of hand and eye movements) vs. absolute normalized distance from the correlation line for each trial. The formats are the same as in Figure 12.


Trial Initiation
Randomized orders
Effector Instruction
Target Instruction
Initial holding (outer) and fixation (innner) zones
Cursor for hand position
Target zones
0.5 s
0.6 s
0.6-1.0 s
Go
<0.5 s
Reaching Initiation
Target Acquisition
0.2-0.5 s
Reward Delivery
Fixation and/or Holding Break
Hand
Eye
Both


A
Hand
Eye
Both
Hand Y
Hand X
Eye Y
Eye X
70 mm
8°
B
Hand
Eye
Both
Hand X
Hand Y
Eye X
Eye Y
70 mm
70 mm
8°
8°
200 ms


Monkey 1
Monkey 2
hand RT
eye RT
ms
100
200
300
400
500


Hand
Both
Eye
Right
Up
Left
Down
1
0.5
0
-0.5
-1
-1000
0
1000
mV
ms

Hand

Both

Eye

A. Hand

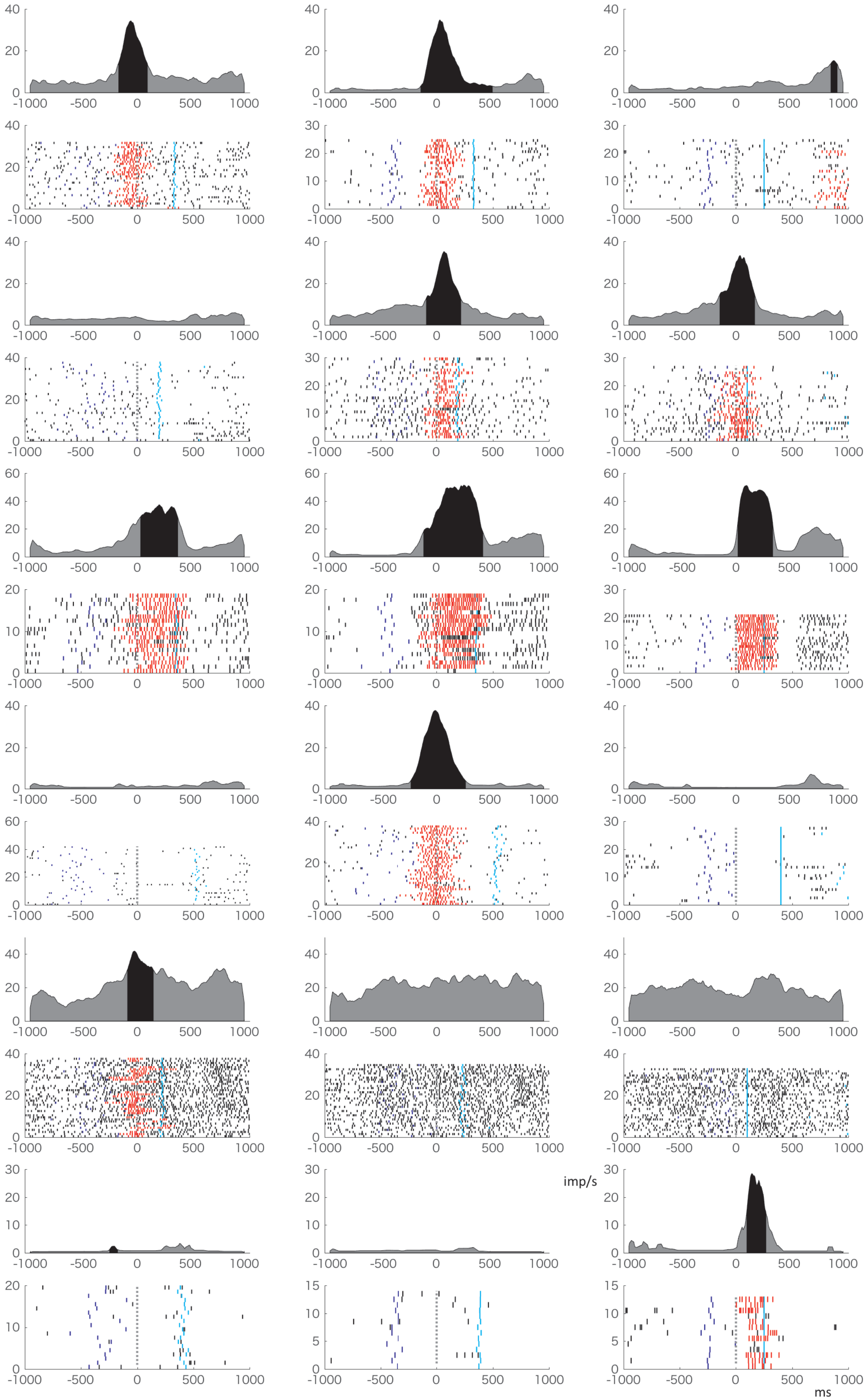
B. Eye

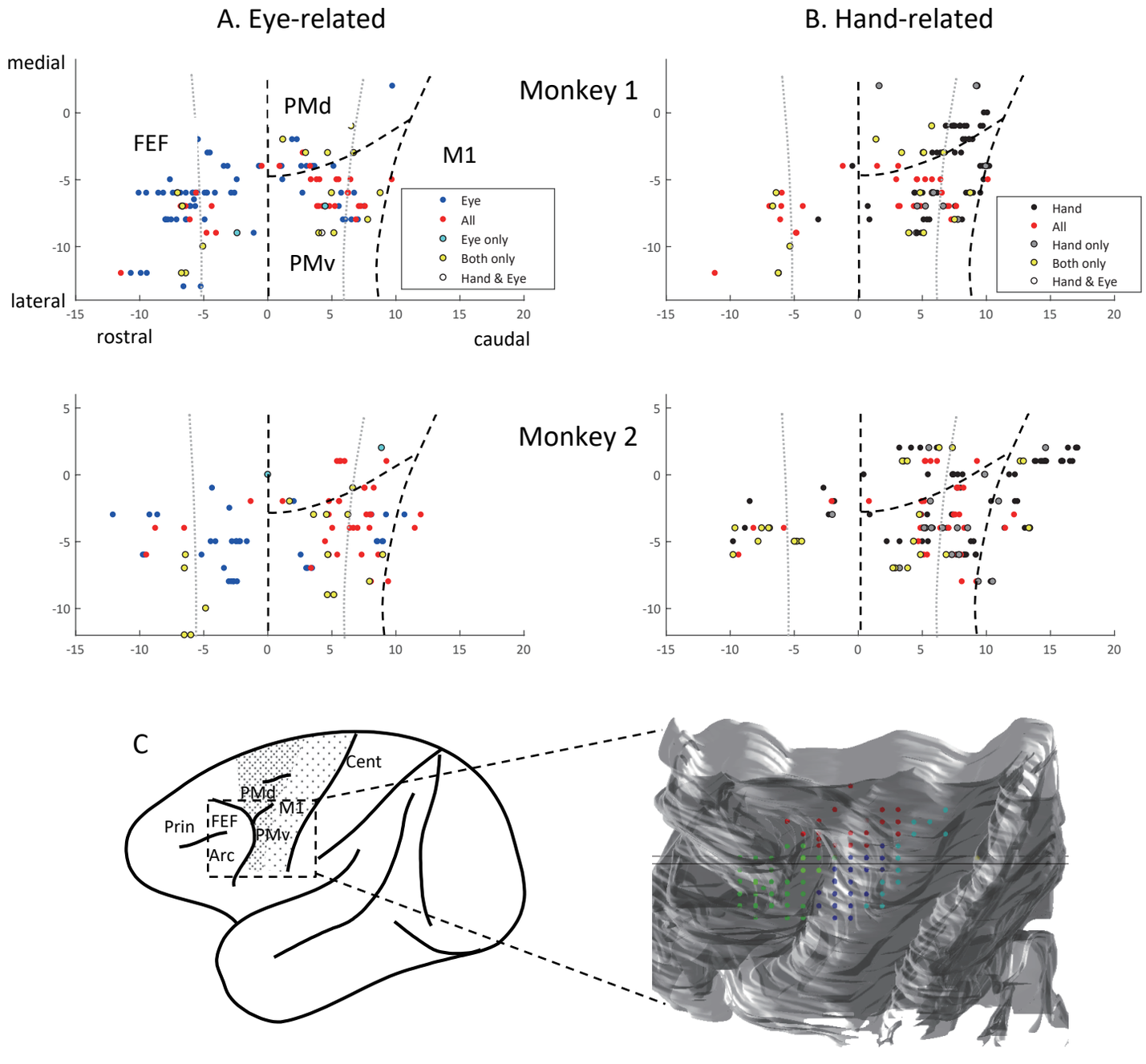
C. All

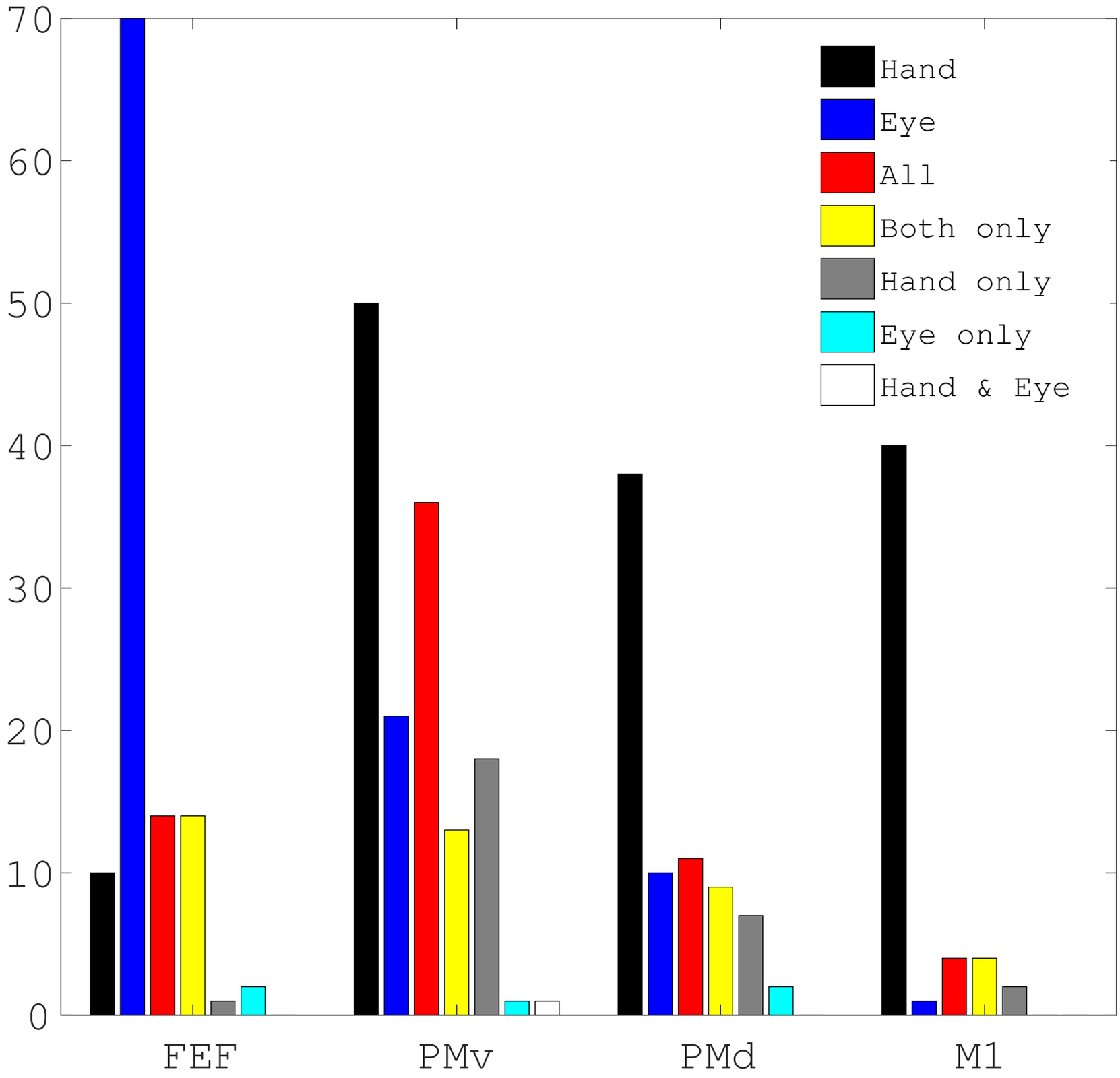
D. Both only

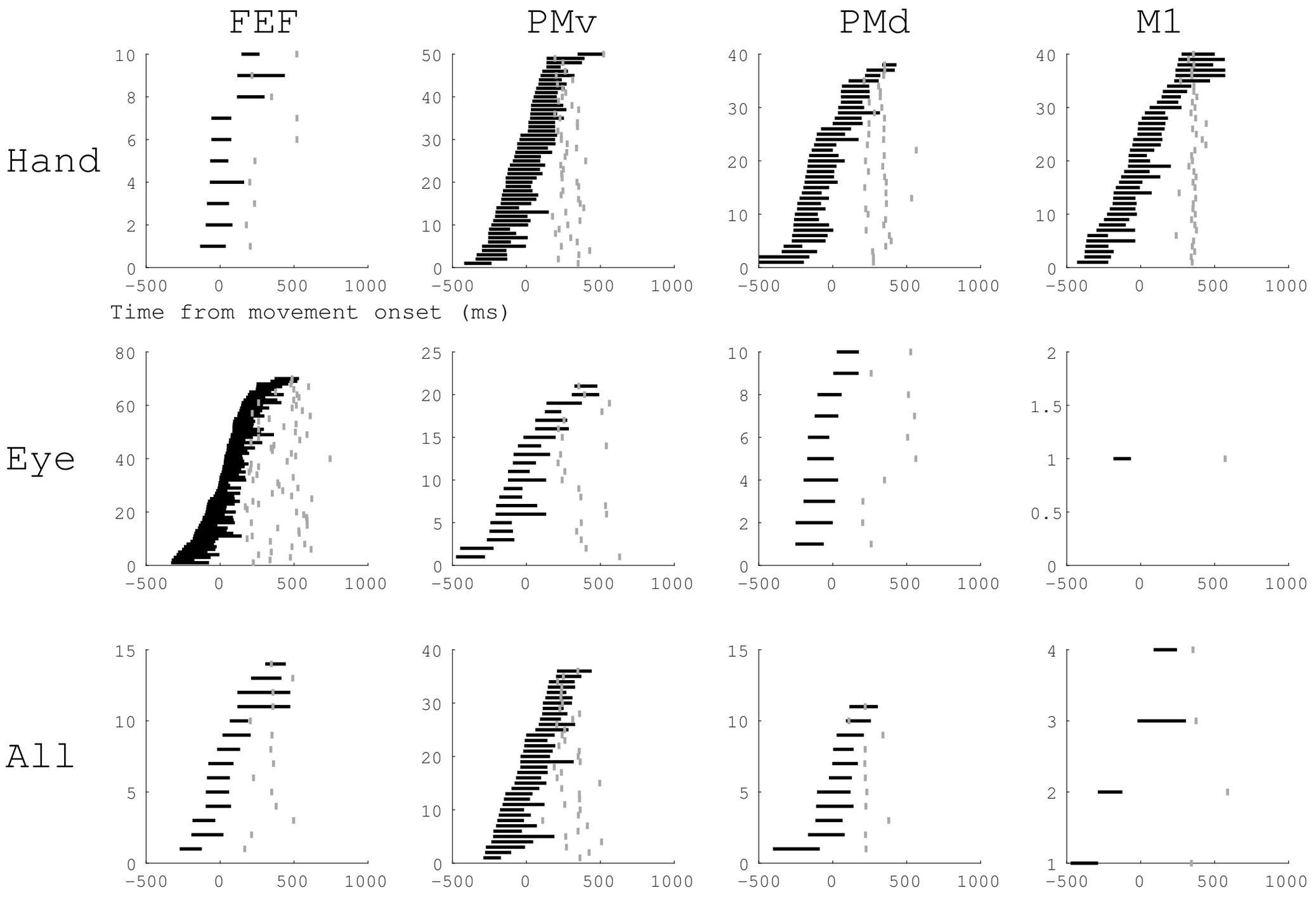
E. Hand only

F. Eye only




A. Eye-related
B. Hand-related
Monkey 1
Monkey 2
medial
lateral
rostral
caudal
FEF
PMd
PMv
M1
Eye
All
Eye only
Both only
Hand & Eye
Hand
Hand only
C
Cent
PMd
M1
Prin
FEF
PMv
Arc


70
60
50
40
30
20
10
0
FEF
PMv
PMd
M1
Hand
Eye
All
Both only
Hand only
Eye only
Hand & Eye


FEF
PMv
PMd
M1
Hand
Eye
All
Time from movement onset (ms)

A

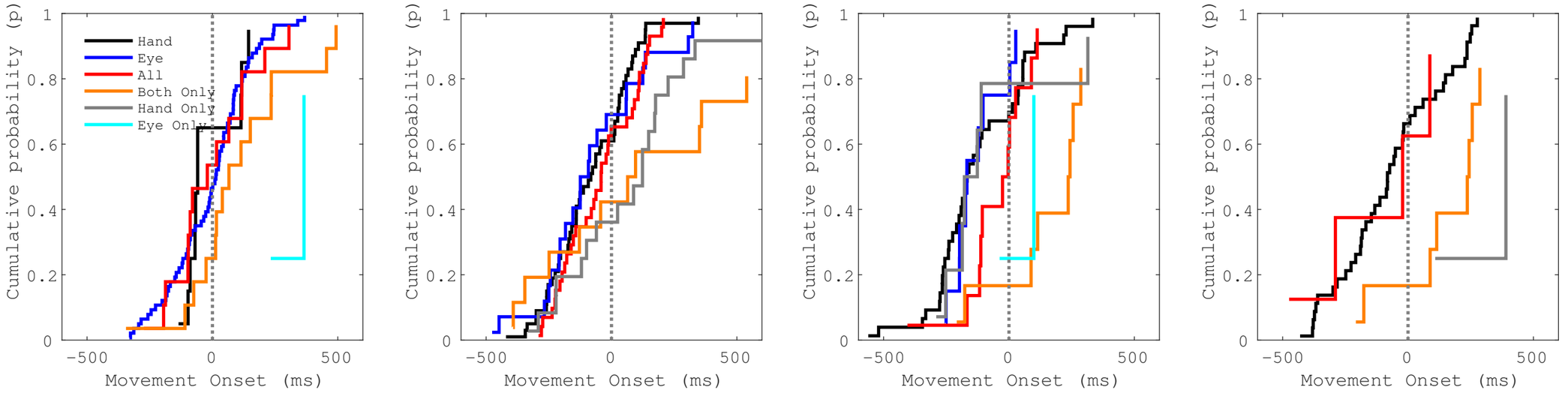
FEF

PMv

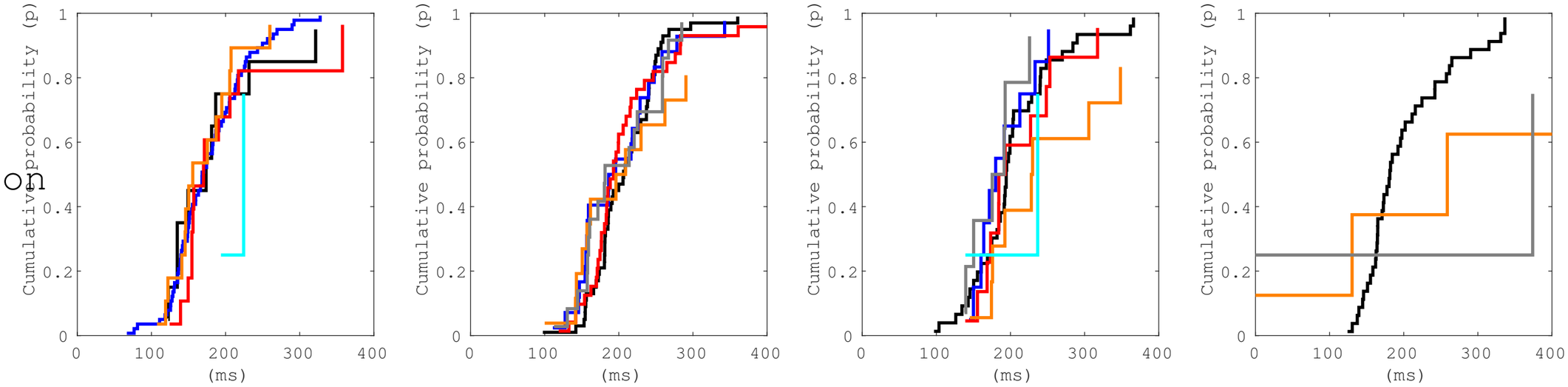
PMd

M1

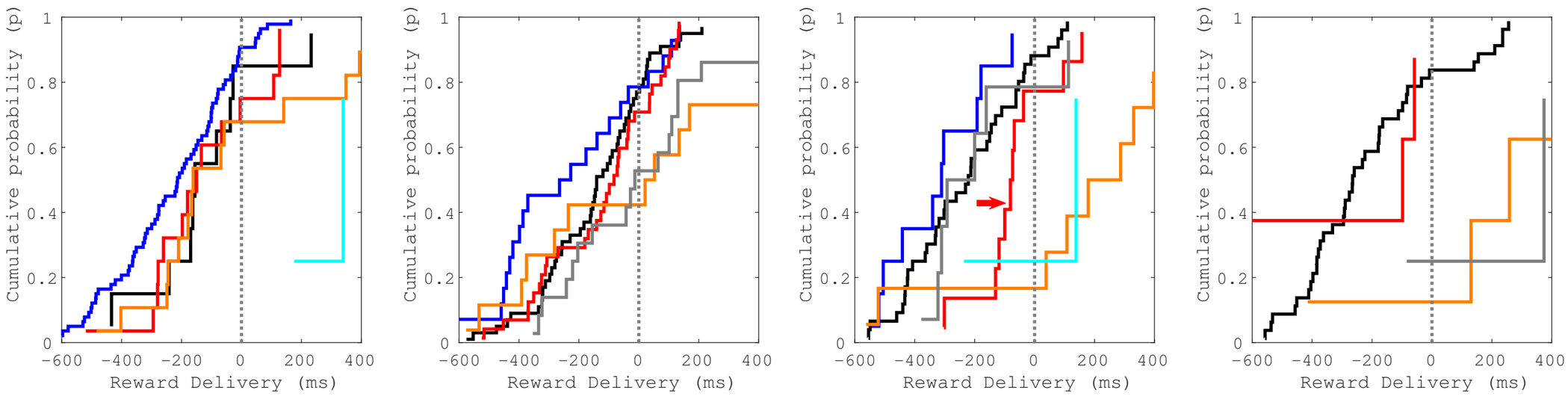
Onset



Duration



Offset



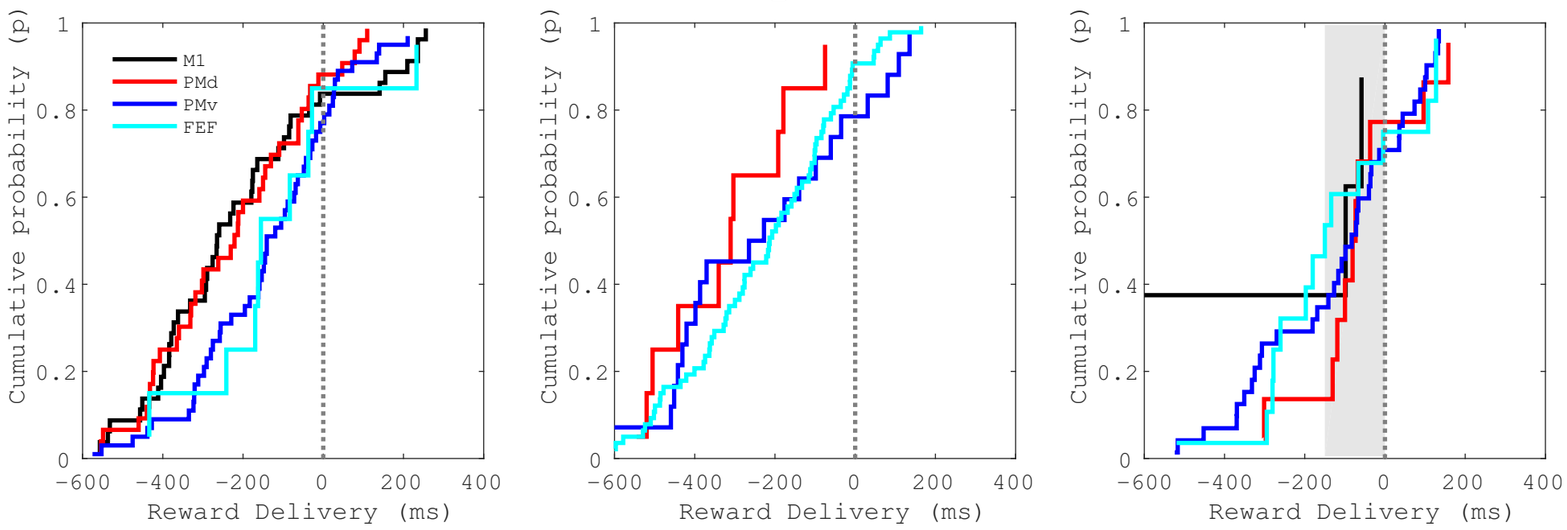
B

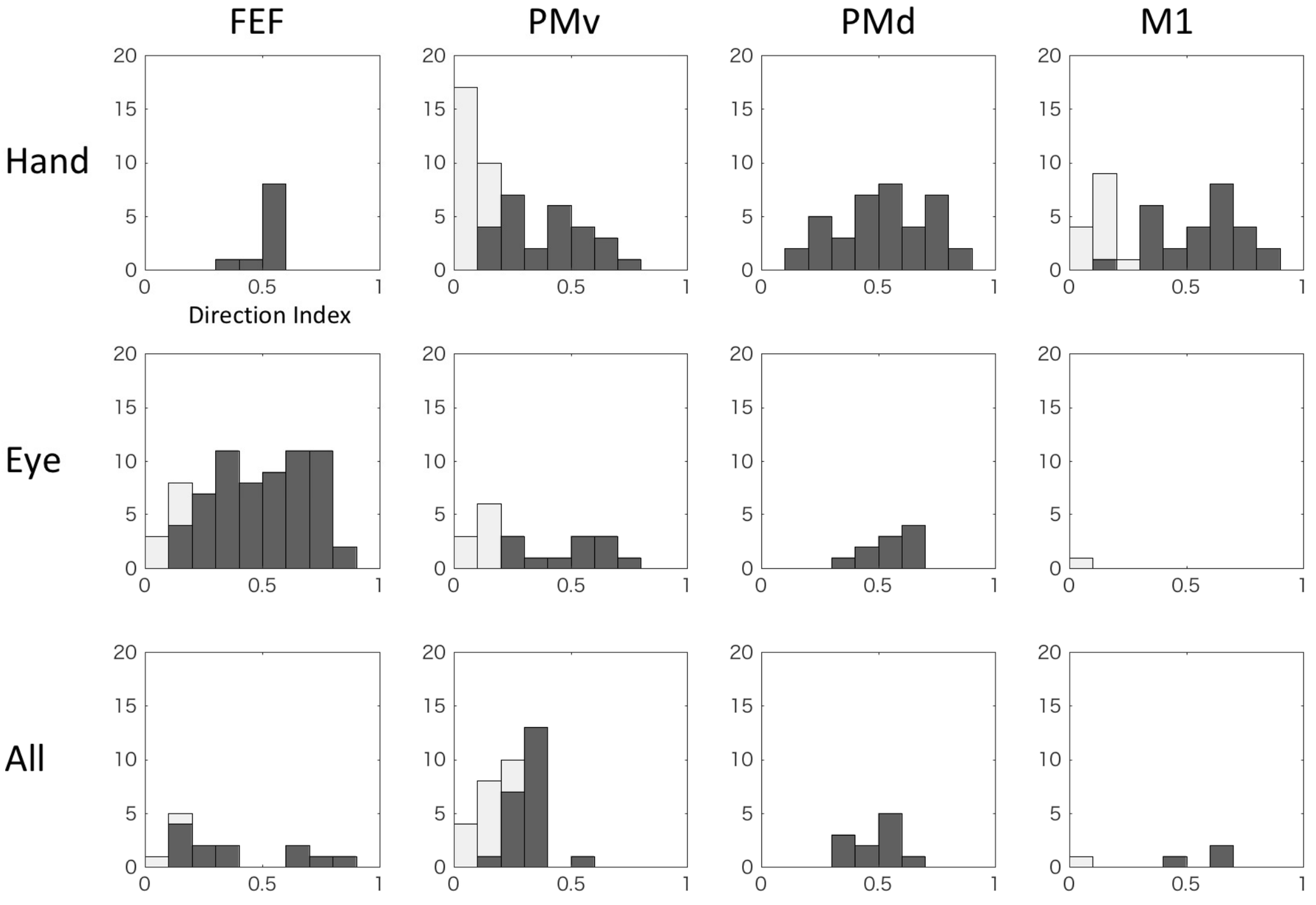
Hand

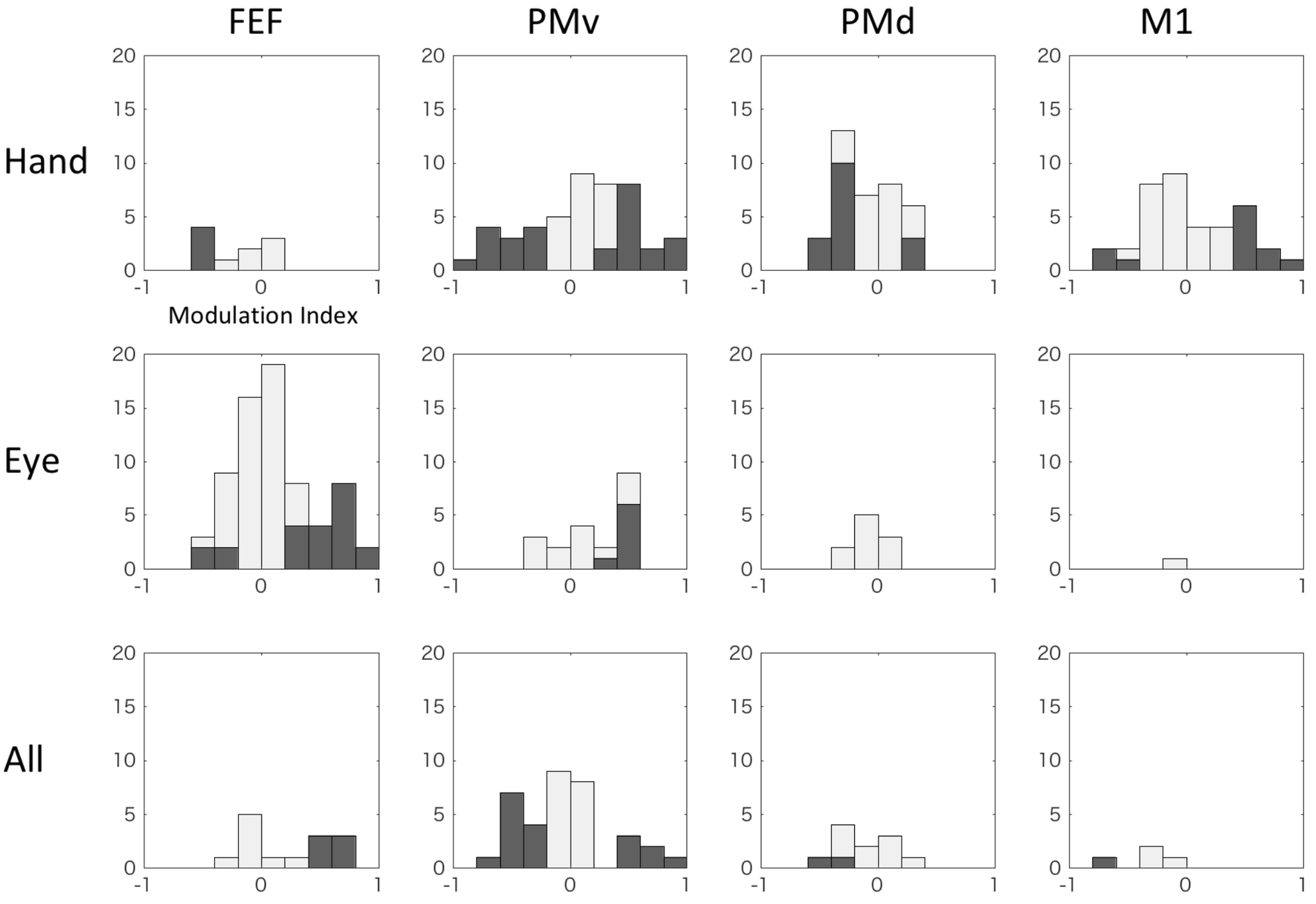
Eye

All

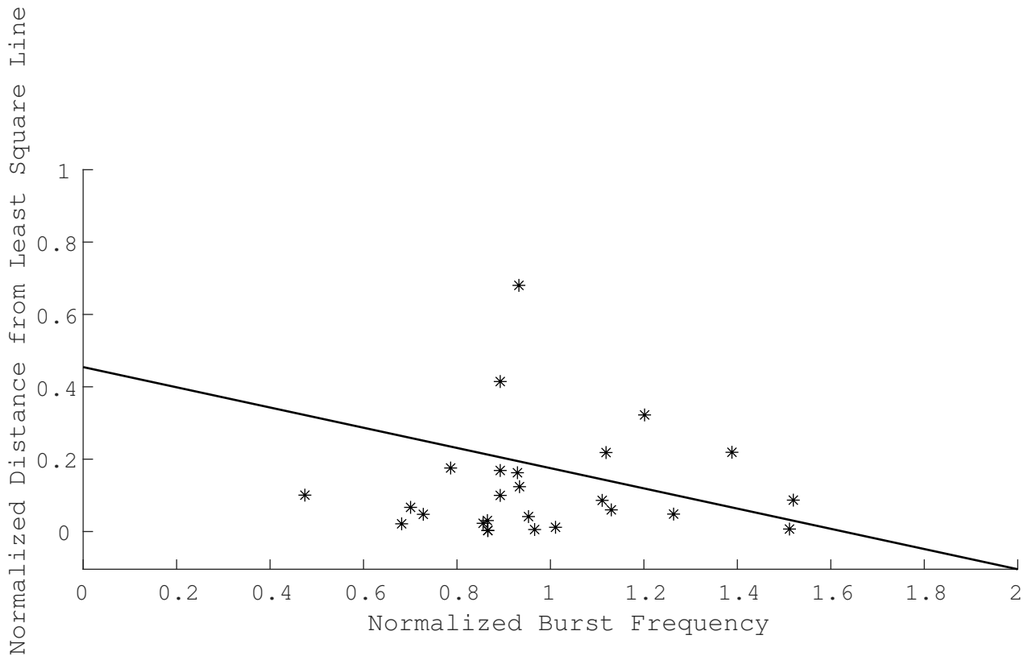
Offset



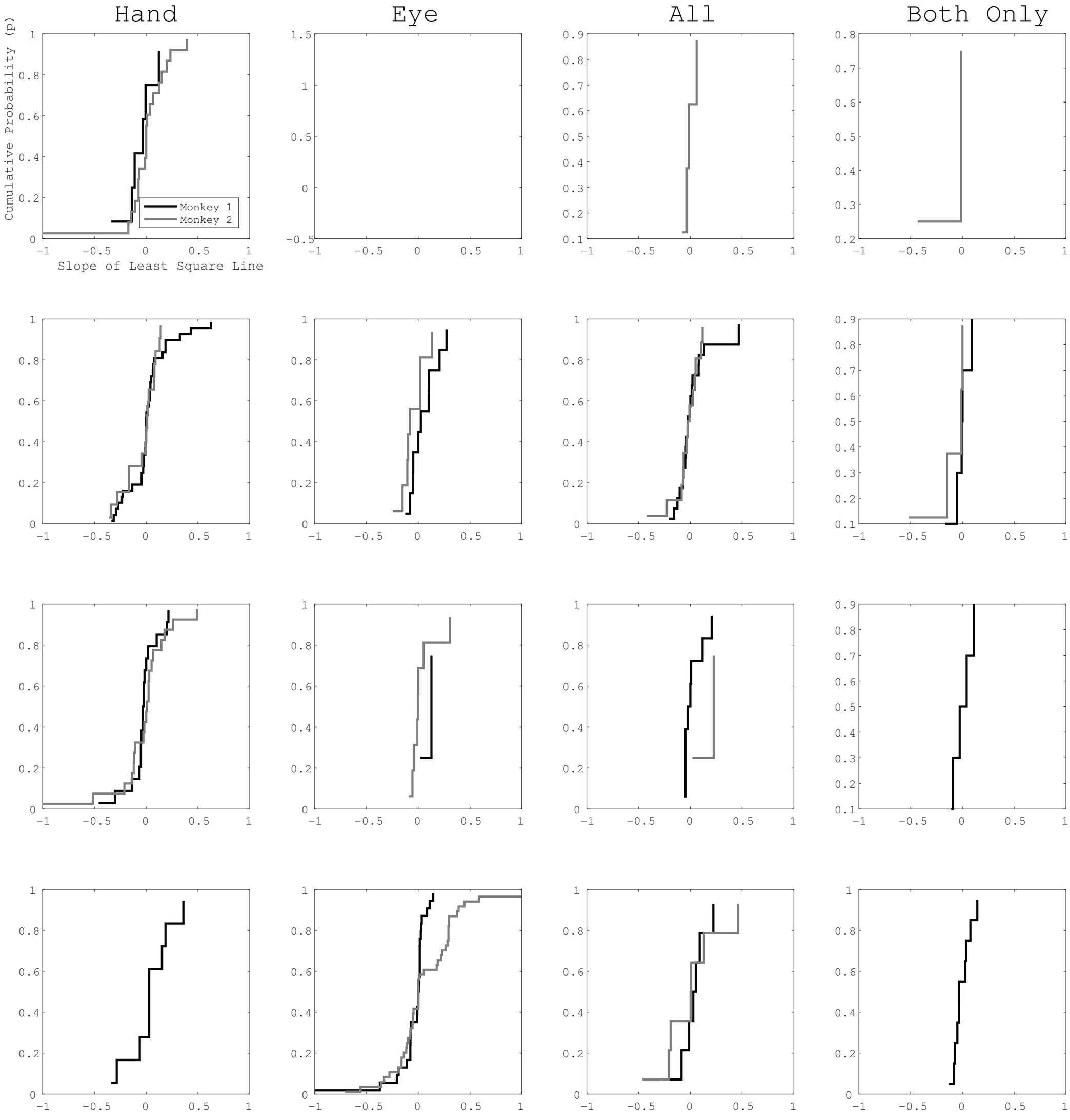

FEF
PMv
PMd
M1
Hand
Eye
All
Direction Index
0
5
10
15
20
0.5
1


FEF
PMv
PMd
M1
Hand
Eye
All
Modulation Index
0
5
10
15
20
-1
0
1

A


Normalized Distance from Least Square Line
Normalized Burst Frequency

B


Hand
Eye
All
Both Only
M1
PMv
PMd
FEF
Cumulative Probability (p)
Slope of Least Square Line
Monkey 1
Monkey 2

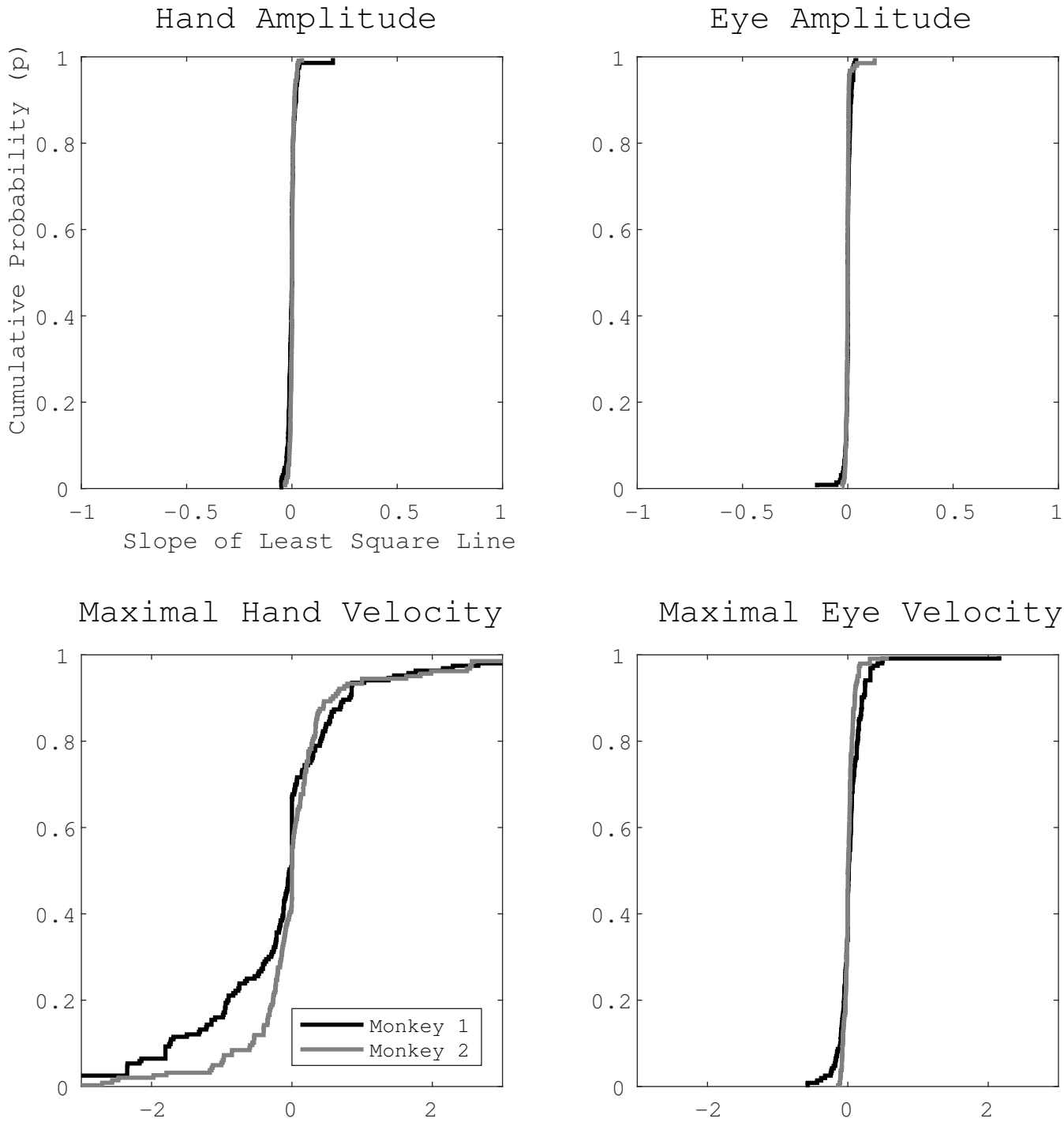

Hand Amplitude
Eye Amplitude
Maximal Hand Velocity
Maximal Eye Velocity
Cumulative Probability (p)
Slope of Least Square Line
Monkey 1
Monkey 2
0
0.2
0.4
0.6
0.8
1
−1
−0.5
0
0.5
1
−2
0
2

Table 1 Reaction and movement times (RT and MT) of eye and hand movements during the three tasks

			Monkey A	Monkey J
RT				
	Eye	Both task	242±52	306±99
		Eye task	241±59	241±71
	Hand	Both task	370±67	310±103
		Hand task	312±94	336±99
MT				
	Eye	Both task	23±9	27±14
		Eye task	15±14	17±20
	Hand	Both task	131±32	162±39
		Hand task	125±19	164±38

Mean and SD of the data are presented in msec.

Table 2. Number of the three major classified neurons with and without preferred direction

	Hand				Eye				All				
Preferred Direction	M1	PMd	PMv	FEF	M1	PMd	PMv	FEF	M1	PMd	PMv	FEF	Total
no	13	23	0	0	1	0	9	7	1	0	14	2	70
right	8	6	11	2	0	1	3	14	0	4	6	3	58
up	7	7	12	2	0	2	1	10	0	2	7	4	54
left	6	8	5	4	0	2	6	23	0	2	5	3	64
down	6	6	10	2	0	5	2	16	3	3	4	2	59