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Spatial eye–hand coordination during bimanual reaching is not systematically coded in either LIP or PRR

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We often orient to where we are about to reach. Spatial and temporal correlations in eye and arm movements may depend on the posterior parietal cortex (PPC). Spatial representations of saccade and reach goals preferentially activate cells in the lateral intraparietal area (LIP) and the parietal reach region (PRR), respectively. With unimanual reaches, eye and arm movement patterns are highly stereotyped. This makes it difficult to study the neural circuits involved in coordination. Here, we employ bimanual reaching to two different targets. Animals naturally make a saccade first to one target and then the other, resulting in different patterns of limb–gaze coordination on different trials. Remarkably, neither LIP nor PRR cells code which target the eyes will move to first. These results suggest that the parietal cortex plays at best only a permissive role in some aspects of eye–hand coordination and makes the role of LIP in saccade generation unclear.

monkey | motor planning | arm movement | saccade | posterior parietal cortex

Eye–hand coordination is critical for natural behavior. Primates normally orient their eyes to the target of their reaches. When a monkey desires to pick an apple hanging from a tree branch, he will look toward the apple before reaching to grasp it. The timing of eye and arm movements is correlated, and this correlation appears to be actively coordinated by specific brain mechanisms rather than arising passively from common input to the two motor systems (1). In human and nonhuman primates, the posterior parietal cortex (PPC) helps transform visuospatial signals into motor commands for the eyes and arms. In humans, PPC damage can result in a constellation of deficits including optic ataxia, the inability to reach for an object under visual guidance, and psychic paralysis of gaze (the inability to saccade to a peripheral target despite intact eye movements) (2). In monkeys, cells in different parts of the PPC encode spatial locations of interest to particular effectors. For example, the parietal reach region (PRR), situated at the posterior end of the intraparietal sulcus (IPS) and overlapping portions of the medial intraparietal area (MIP) and V6a, contains cells that encode the direction or endpoint of an upcoming reach (3–5), particularly for the contralateral arm (6, 7). Similarly, the lateral intraparietal area (LIP), located midway along the lateral bank of the IPS, contains cells that encode the direction or endpoint of an upcoming saccade (8–13). These LIP cells are active when a saccade is planned into the response field (RF), less active when a dissociated reach is planned (a reach without an accompanying saccade), and still less active for a reach into the RF combined with a saccade out of the RF (3, 10, 14). Lesion data further support the idea that PRR preferentially codes reaches while LIP preferentially codes saccades (15–21).

Evidence is mixed regarding the involvement of these areas in eye–hand coordination. LIP responds to some extent when a reach is made without an accompanying saccade, and, similarly, PRR responds when a saccade is made without a reach (22). Furthermore, LIP cells respond differently to coordinated saccades and reaches than to saccades alone (23). In both areas, local

field potential power in the beta-frequency band predicts the reaction times of eye movements coordinated with a reach but not of isolated eye movements (24). Cells in the posterior IPS that fire coherently with beta-frequency local field potentials encode the direction of upcoming combined reach-plus-saccade movements (25). These findings are all consistent with a role of parietal areas in eye–hand coordination. However, reversible inactivation of LIP does not affect eye–hand coordination, and inactivation of PRR produces mixed results (16, 19, 21).

Eye–hand coordination is difficult to study because coordinated eye–hand behavior is highly stereotyped. Isolating the activity specifically related to coordination requires breaking this stereotypy. Animals may be trained to move the eyes to a different target or at a different time than the reach or to perform a reach without moving the eyes at all (1, 3). Each of these altered patterns of movement, however, requires considerable practice to accomplish reliably. By overtraining novel patterns of activity that circumvent natural coordination, we risk bypassing the very circuitry we wish to study.

Here we take a different approach to this problem. Consider a monkey picking an apple while reaching with the other hand to grasp a nearby branch to stabilize itself. Which target will it look at first, the apple or the branch? To address the programming of eye and arm movements to the same spatial location, we trained animals to make bimanual (two-arm) movements to two targets, without constraining eye movements during the movement period. Animals typically look first at one target and then at the other. On some trials, an animal chose to look first at the target of the right arm, and on other trials it chose to look first at the target of the left arm. Since LIP has been shown to encode the very next movement to be made, we expected that, if LIP is involved in eye–hand coordination, then its activity during the delay period before the first saccade will depend on this choice (9, 26). In PRR, the story is less clear, but PRR in each

Significance

When we reach for something, we also look at it. If we reach for two objects at once, one with each hand, we look first at one and then the other. It is not known which brain areas underlie this coordination. We studied two parietal areas known to be involved in eye and arm movements. Neither area was sensitive to the order in which the targets were looked at. This implies that coordinated saccades are driven by downstream areas and not by the parietal cortex as is commonly assumed.

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hemisphere encodes primarily the movement of the contralateral arm (27–29). Therefore, we expected that, as with LIP, if it is involved in eye–hand coordination, then its activity during the delay period before the first saccade will depend on whether the eyes will move with the contralateral or ipsilateral arm. However, we find no differential effects in either area. Neither region systematically specifies the direction of a coordinated saccade during a bimanual reach, suggesting that this aspect of eye–hand coordination may be mediated outside the PPC.

Results

We recorded single-unit responses from 89 PRR and 64 LIP cells from both hemispheres in each of two monkeys (M1 and M2) during reach and saccade tasks (Fig. 1 *A* and *B*). As expected, cells in the two regions behaved very differently (Fig. 1 *C* and *D*). LIP cells showed similar modulation for saccade-only, contralateral arm reach-plus-saccade, and ipsilateral arm reach-plus-saccade trials [15.52 ± 1.66 , 16.60 ± 1.86 , and 14.32 ± 1.59 spikes per second (sp/s), respectively, each $P < 0.001$], with no difference across conditions [one-way ANOVA of mean modulation, $F(2, 63) = 2.00$, $P = 0.14$]. PRR cells, in contrast, showed greater modulation for contralateral compared with ipsilateral arm reach (25.55 ± 1.87 versus 11.34 ± 1.37 sp/s, significant at $P < 0.05$ for 69% of the 89 individual cells) or compared with saccade-only trials (13.26 ± 1.57 sp/s, significant for 73% of 89 cells). Thus, as previously described, LIP encodes primarily the presence or absence of a saccade into the RF, while PRR primarily encodes a contralateral arm reach into the RF, with about half as much modulation for effectors other than the contralateral arm (6).

Next, we considered spatial patterns of eye–hand coordination. During the delay period, animals were required to fixate straight ahead. For single-target trials, saccades to the reach target were required, reinforcing the natural behavior (30, 31). However, in “bimanual-apart” trials, in which one arm was cued to a target in the RF of a recorded cell and the other arm was cued to a target on the opposite side of the fixation point, the eyes were unconstrained once the go cue was delivered. The timing of saccade and reaches for each animal over all trials is shown in Fig. S1. Despite being unconstrained, a coordinated saccade was made to one of the two reach targets in 99% of trials. In 44% of trials, a second saccade was made directly to the other target, on average 227 ms after the first saccade. The first saccade began before either reach, and the second saccade, when it occurred, began after both reaches were initiated. The two reaching movements were tightly coupled in time whether sorted by movement order (first, second), arm identity (left, right), or movement direction (left, right). For a given pair of targets, the two possible arm configurations (instructed) and the two possible initial saccade directions (freely chosen) resulted in four possible patterns of spatial eye–hand coordination (Fig. 2*A*). We computed how often the first saccade accompanied the left versus the right arm, accompanied the arm that moved first versus second, or went to the left versus the right target (Table 1). Choices were rarely perfectly stochastic (ratios of 1:1) but also were far from deterministic (ratios of 1:0 or 0:1), with a maximum imbalance of 3:1. Table S1 shows a finer categorization of these data. These biases occur in absolute space. Cells were obtained from both hemispheres of both animals, so when expressed relative to the side of the recording or into or out of a cell’s RF, biases are much reduced (Table S2). Finally, by considering only those cells for which at least two saccades were made into and out of the RF across bimanual-apart trials, we reduce bias in the saccade direction even further (Fig. S2).

Fig. 2*B* shows a set of scan paths obtained using a single-arm configuration (right arm to the upper right and left arm to the lower left) while recording from one cell. After the initial saccade directed to one or the other reach target (light blue and green traces), a second saccade was made to the other reach target (dark traces). This resulted in just two highly repeatable

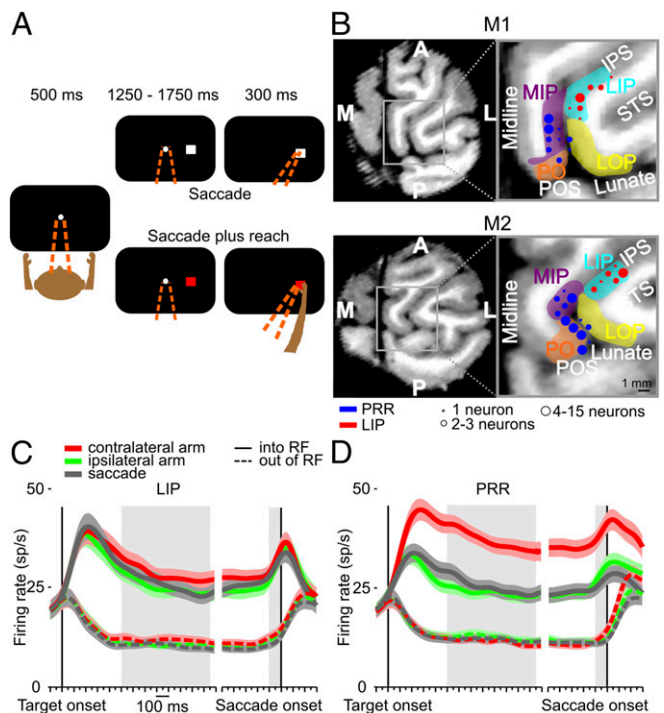


Fig. 1. (A) Delayed-movement tasks. A peripheral target instructed the spatial location and effector to be used (eyes or arm) for each trial. The stimulus remained visible during the delay period. In the primary task, two stimuli appeared separated by 180° across the fixation. At the end of a variable delay period, the central target was extinguished, cueing the animal to reach with the right arm to the red target (shown) and with the left arm to the green target. Interleaved with this principle bimanual-apart task were four other trial types. Unimanual left or right arm (*Lower Row*) reaches were instructed with a single green or red peripheral target, respectively. Reaches with both arms to a single target (bimanual-together task) were instructed with a blue target, and saccade-only trials (no reach) were instructed with a white target (*Upper Row*). Throughout saccade and unimanual reach trials, the hand(s) that were not instructed to move were required to remain on the home pad(s). On unimanual reach trials, eye movements to the target of the reach were required. On bimanual reach trials, eye movements were unconstrained once the animals were cued to initiate the movement. Movements were either into or 180° out of the RF. Movement directions and movement types were randomly interleaved. (B) Recording sites from the right hemisphere of each monkey. Coordinates of recorded cells in M1 (*Upper Row*) and M2 (*Lower Row*) are shown projected to a single MRI section perpendicular to the path of the recording electrode, with zoomed-in views on the right. IPS, intraparietal sulcus; LIP, lateral intraparietal area; LOP, lateral occipital–parietal area; Midline, longitudinal fissure; MIP, medial intraparietal area; PO, parieto-occipital area; POS, parieto-occipital sulcus; STS, superior temporal sulcus. The colored regions are from ref. 70. The left, right, anterior, and posterior directions are labeled as L, R, A, and P, respectively. Red circles indicate LIP cells; blue circles indicate PRR cells. The size of each circle indicates the number of cells recorded along that track. (C and D) Population average firing rates (mean \pm SEM) as a function of instructed effector and movement direction for saccades and unimanual arm reaches shown aligned to target onset (*Left*) and saccade onset (*Right*) for LIP (C) and PRR (D). Solid lines denote movements into the RF, and dashed lines denote movement out of the RF. Gray shading indicates the intervals used to measure spiking activity during the delay and presaccadic periods. (C) Across the population, LIP cells respond when the animal prepares a saccade to a target in the RF. There is no difference in modulation among movement conditions [$n = 64$ cells; one-way ANOVA of mean modulation, $F(2, 63) = 2$, $P = 0.14$]. Activity is suppressed, relative to baseline, for any movements out of the RF. (D) Across the population, PRR cells respond when the animal prepares a contralateral arm reach (solid red trace) to a target in the RF. The contralateral arm response is greater than the response when the animal prepares a saccade (solid gray trace; $P < 0.001$) or saccade plus ipsilateral arm reach (solid green trace; $P < 0.001$) to a target in the RF. Activity is suppressed, relative to baseline, for any movements out of the RF.

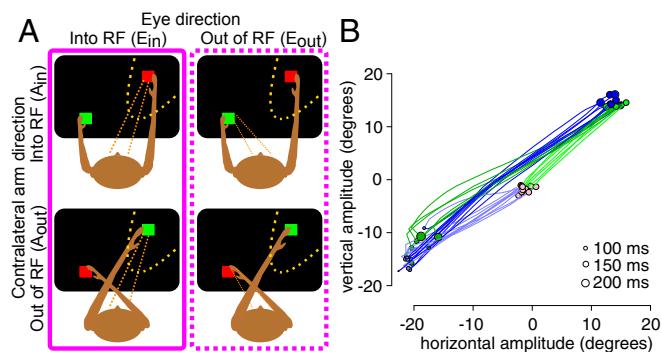


Fig. 2. Eye–arm movement direction combinations on two-target delayed bimanual movement tasks. (A) On two-target trials, bimanual movements were made with each arm to a different target. The two-target reach task has the same task structure as the single-target tasks, except that two targets appear and instruct a different reach for each arm (green, left arm; red, right arm). The two targets are diametrically opposed about the fixation point, one in the RF (dotted yellow arc), and one out of the RF. Eye movements are unconstrained, but almost all were directed to the target of either the left or the right arm (dotted orange lines). The natural variability in saccade behavior results in four eye–arm combinations: contralateral arm in, eyes in; contralateral arm in, eyes out; contralateral arm out, eyes in; and contralateral arm out, eyes out. The borders indicate the colors and line types used to denote each condition in subsequent figures. Solid magenta: eyes into the RF; dashed magenta: eyes out of the RF. (B) Typical eye movement trajectories (scan paths) for a single cell. Initial saccades were either up and right (light green) or down and left (light blue). A second saccade was then made to the diametrically opposed target (darker green and blue). Circles represent fixations at the beginning of the trial (pink) and saccade endpoints (blue and green), with diameters proportional to fixation duration.

patterns of spatial coordination. As previously noted, eye–hand coordination arose naturally, since no constraints were imposed on the eyes once the go cue was delivered. For this particular animal and target configuration, the initial saccade was directed to the upper-right target in 63% of trials and to the lower-left target in 37% of trials. For other target configurations, the proportions could be different, or the initial saccade might always be in the same direction, resulting in just one scan path.

In addition to being spatially coordinated, saccades and reaches were also temporally coordinated. Correlation of eye reaction time and reach reaction time, a standard measure of eye–hand coordination (32), was high in unimanual reach tasks (Table 2). Correlation was also high when both arms went to a single target (bimanual together), computed either for the arm that moved first or for the arm that moved second. For bimanual-apart tasks, eye–arm correlation depended on whether the correlation was computed using the arm that moved with or opposite the eyes and on whether that arm moved first or second. In every case, however, there was substantial temporal coordination (correlation coefficients >0.5). A full treatment of the patterns of eye–hand coordination has been previously published, using different data but showing similar effects (33). In the present study, we focused on the spatial coordination of saccades and reaches and asked how the direction of the first saccade affected neural activity. An area involved in coding eye–hand coordination would, by definition, show differences in activity for different patterns of eye–hand coordination and, in particular, for different saccade directions during bimanual-apart trials.

LIP. LIP cells encode upcoming eye movements more strongly than arm movements made without a saccade (3, 10, 14, 34). Furthermore, in a dual-movement task with a simultaneous reach and saccade in opposite directions, LIP cells encode the saccade, not the reach (3, 35). We did not train our animals to perform dissociated unimanual reaches, since such training might conceivably alter eye–hand coordination on unimanual or

bimanual-apart reaches. However, the clear results from dual-movement opposite-direction tasks, combined with studies showing that LIP codes the very next saccade in a sequence of saccades (9, 26), set a strong expectation that LIP activity during the delay and pre-movement periods should reflect the direction of the first saccade. We therefore predicted higher activity in bimanual-apart trials when the saccade moves into (versus out of) the RF, regardless of arm configuration: $E_{in}A_{in}$ higher than $E_{out}A_{in}$, and $E_{in}A_{out}$ higher than $E_{out}A_{out}$, where E represents the saccade and A represents the arm movement. However, this is not what we found.

Fig. 3A shows the effect of saccade direction on an exemplary LIP cell for bimanual-apart (magenta traces) and unimanual (black traces) reaches. The responses are aligned on target onset (Left) and on the saccade onset (Right). The delay-period activity was high when the animal chose to saccade into the RF (E_{in} ; solid magenta trace, $n = 12$ trials, 43.07 ± 4.62 sp/s). Surprisingly, delay activity was also high when the animal chose to saccade away from the RF (E_{out} ; dashed magenta trace, $n = 3$ trials, 41.70 ± 4.40 sp/s; Wilcoxon rank-sum test, $P = 0.83$). Activity then increased to similar levels just before a saccade in either direction (Right, E_{in} versus E_{out} , 52.80 ± 4.83 versus 45.49 ± 6.10 sp/s; Wilcoxon rank-sum test, $P = 0.49$). In comparison and as expected, activity was high for a unimanual reach plus coordinated saccade into the RF (solid black trace; $n = 30$ trials, 37.51 ± 2.38 and 47.26 ± 3.76 sp/s for the delay and presaccadic periods, respectively) and low for a unimanual reach plus saccade out of the RF (dashed black trace; $n = 30$ trials; 17.97 ± 2.12 and 15.84 ± 1.87 sp/s, for delay and presaccadic periods, respectively). In both time intervals, both E_{in} and E_{out} bimanual responses were substantially greater than the unimanual out-of-RF response (Wilcoxon rank-sum tests; delay, E_{in} : $P < 0.001$; E_{out} : $P = 0.009$; presaccadic, E_{in} : $P < 0.001$; E_{out} : $P = 0.006$), and neither bimanual response was significantly different from the unimanual in-RF response (Wilcoxon rank-sum tests; delay, E_{in} : $P = 0.21$; E_{out} : $P = 0.43$; presaccadic, E_{in} : $P = 0.93$; E_{out} : $P = 1.00$). This pattern of activity is inconsistent with coding the direction of the coordinated saccade.

Similar results held at the population level. Fig. 3B shows the average time course for 29 cells recorded when the animal chose to make at least two saccades into and at least two saccades out of the RF on bimanual-apart trials with identical arm instructions, i.e., either the upper or lower row in Fig. 2A. The median number of trials was nine. Across the population of LIP cells, mean firing rates were nearly identical for E_{in} and E_{out} saccade directions during the delay (21.45 ± 3.09 versus 22.60 ± 3.00 sp/s; $P = 0.18$) and presaccadic period (25.86 ± 3.23 versus 26.43 ± 3.68 sp/s; $P = 0.37$). Firing rates diverged only after saccade onset. The in-RF and out-of-RF responses ramped up at the same rate before saccade onset, with the in-RF response peaking at saccade onset while the out-of-RF response continued to rise and peaked ~ 100 ms after saccade onset. Black traces in Fig. 3 show the responses for unimanual in-RF (solid traces) and out-of-RF (dashed traces) reaches during the delay (25.57 ± 3.08 and 12.28 ± 1.83 sp/s, respectively) and presaccadic periods (31.96 ± 2.93 and 16.23 ± 2.51 sp/s, respectively). Bimanual population

Table 1. Behavioral biases: Percentage of trials in which the first saccade moved to the target of a particular arm based on arm identity, movement order, or movement direction

Percentage first saccades	Animal	
	M1	M2
Accompanying left (right) arm, %	28 (72)	48 (52)
Accompanying first (second) arm, %	58 (42)	75 (25)
Directed to left (right) target, %	65 (35)	44 (56)

Table 2. Eye–hand temporal coordination correlation values

Condition	Pearson's <i>r</i>
Unimanual	0.65
Bimanual together (one target)	
Correlation with arm that moves first	0.69
Correlation with arm that moves second	0.62
Bimanual apart (two targets): Correlation with arm that:	
Moves with eyes and moves first	0.61
Moves opposite the eyes and moves first	0.57
Moves with eyes and moves second	0.54
Moves opposite the eyes and moves second	0.54

Note that temporal correlation is at least 80% as high in the bimanual tasks as in the unimanual task.

responses for the E_{in} and E_{out} directions were much greater than unimanual out-of-RF responses in the delay and presaccadic periods (all $P < 0.001$), slightly less than the unimanual in-RF response during the delay ($P = 0.03$ and 0.05 , respectively), and similar to the unimanual in-RF response just before the saccade ($P = 0.24$ and 0.06 , respectively).

Remarkably, none of the 29 individual cells showed significantly greater activity in the E_{in} compared with the E_{out} condition during the delay period (Fig. 3C). One cell showed significantly less activity in E_{in} than E_{out} ($P < 0.001$). Immediately before the saccade, 3 of the 29 cells showed significantly different ($P < 0.05$) activity in the E_{in} compared with the E_{out} condition (two cells: $E_{in} > E_{out}$; one cell: $E_{in} < E_{out}$) (Fig. 3D), a number not significantly greater than that expected by chance (binomial test, $P = 0.18$).

After saccade onset, the peak response for movements out of the RF occurred ~ 100 ms after the peak response for movements into the RF (dashed vs. solid traces) (Fig. 3B). Since two targets on opposite sides of the fixation remained on the screen throughout the trial, this delayed response is consistent with either a movement intention or an early motor response to move either back to fixation or, in some cases, to the other target. The effect is not observed when the animal first saccades to the in-RF target since after the eyes move there is no longer a target in the RF.

The magnitudes of the increases in E_{in} and E_{out} responses compared with their delay-period activity were similar to the unimanual in-RF response 50 ms after saccade onset, differing by 2 sp/s in each case. Differences among conditions only emerged later, as was appropriate for the updated sensory and motor context of the cell after the initial saccade was made. Over the 50- to 150-ms period after saccade onset, 8 of the 29 cells showed significantly different ($P < 0.05$) activity in the E_{in} compared with the E_{out} condition (two cells: $E_{in} > E_{out}$; six cells: $E_{in} < E_{out}$) (Fig. S3).

Because animals chose the saccade direction freely, the number of trials acquired for a given saccade direction can be quite low. These small numbers limit our ability (power) to detect differences between the two conditions at the single-cell level. However, the small error bars for most cells (Fig. 3C and D) suggest that trial-to-trial variability per se does not explain the lack of significant effects. Furthermore, raising the criterion number of trials does not affect the results. With a minimum of four trials in each direction, for example, delay activity differs by only 1.43 ± 1.08 sp/s ($P = 0.19$, $n = 19$), and presaccadic activity differs only by 0.14 ± 2.40 sp/s ($P = 0.95$, $n = 19$) as a function of saccade direction. With a minimum of six trials, 1 of 11 cells showed a significant ($P < 0.05$) effect of saccade direction in the delay period, and 2 of 11 cells showed a significant ($P < 0.05$) effect of saccade direction in the presaccadic period.

The single-cell data are suggestive but not definitive. However, we had a very strong prior expectation, based on all previous work in LIP, for a systematic population effect, i.e., higher activity when the saccade is directed into rather than out of the RF. Our data provide ample power to rule out this expected systematic effect.

This can be seen in the single-cell analyses of Fig. 3C and D, where a regression line fit to the data provides strong evidence against a larger population response in the E_{in} condition than in the E_{out} condition. Finally, pooling across all 64 of our cells reveals no difference in activity between E_{in} and E_{out} in the delay period (20.63 ± 2.60 versus 19.11 ± 2.14 sp/s, $P = 0.65$) or immediately before the saccade (24.48 ± 3.1 versus 20.52 ± 2.44 sp/s, $P = 0.34$).

A change in plan does not explain why saccade direction is not encoded. One possible explanation for not coding saccade direction would be that animals do not settle on a particular saccade direction until late in each trial. If, on each bimanual-apart trial, the animal changed plans multiple times over the course of the delay period, then we would expect the activity averaged over many trials to lie midway between the activity evoked for unimanual movements into and out of the RF. This was not the case. The mean bimanual-apart delay-period activity (22.03 ± 3.01 sp/s) was greater than the mean of the average activity in the unimanual conditions (18.92 ± 2.29 sp/s; one-sided, $P = 0.012$). More correctly, we can account for directional biases by weighting the two unimanual conditions by the proportions of saccades made in each direction. The mean bimanual-apart activity remained greater than the unimanual weighted mean, although the difference did not reach significance (19.79 ± 2.85 sp/s; one-sided, $P = 0.06$). One would also predict greater

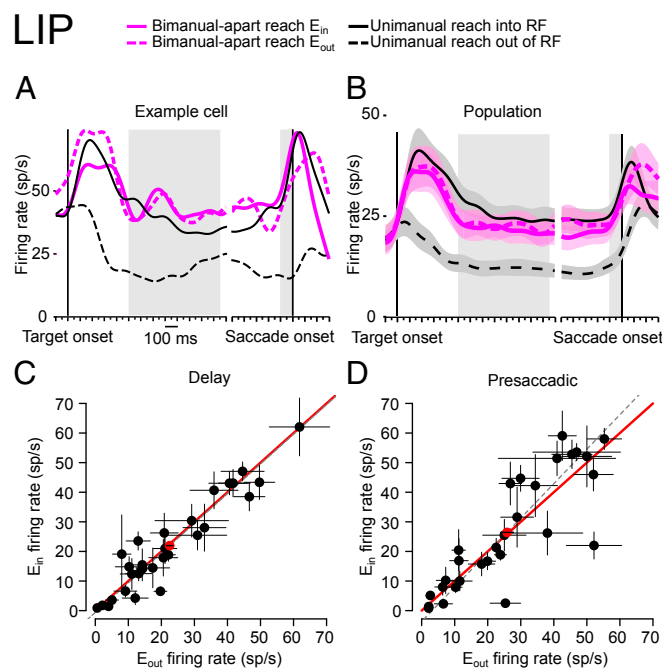


Fig. 3. LIP cells do not encode saccade direction during bimanual reaches to two separate targets. (A) Average responses of an exemplary LIP cell for the bimanual-apart (magenta traces) and unimanual reach-plus-saccade (black traces) trials. Solid lines denote saccades into the RF (E_{in}); dashed lines denote saccades out of the RF (E_{out}) (Fig. 2). Responses are shown aligned to target onset (Left) and saccade onset (Right). Gray shading indicates the interval used to measure delay (Left: 500–1,250 ms) and presaccadic (Right: 100–0 ms) activity. Responses did not vary as a function of saccade direction ($n = 12$ E_{in} trials, 3 E_{out} trials; Wilcoxon rank-sum test: delay, $P = 0.83$; presaccadic, $P = 0.49$). (B) Population average (mean \pm SEM) activity of LIP cells. The format is the same as in A. Data are from all cells for which there were at least two trials of each saccade direction. Responses did not vary as a function of saccade direction ($n = 29$ cells; Wilcoxon signed-rank test: delay, $P = 0.45$; presaccadic, $P = 0.37$). (C and D) Scatterplots of the firing rates for E_{in} vs. E_{out} bimanual-apart reaches for the cells shown in B. Each point represents a single cell in the delay period (C) and presaccadic period (D). Error bars indicate the SEM. The unity line is in red. The dashed gray line is a type-II regression line.

variability during two-target bimanual-apart trials compared with single-target trials (saccade-only, ipsilateral arm, contralateral arm, and both arms together). We computed the coefficient of variation (CV) as the mean SD divided by mean interspike interval, by condition and by cell. The CV was not larger for bimanual-apart versus single-target trials across the 29 cells ($P = 0.85$ for E_{in} trials and $P = 0.21$ for E_{out} trials).

Effects of freely chosen saccade direction in LIP. A failure to code future saccade direction in the bimanual-apart reaching task could reflect something special about saccades that accompany reaches (eye–hand coordination), or it could reflect a general failure of LIP to code saccade direction when the animal is presented with two targets and is free to choose between one of two movement plans. In fact, several studies have shown equal encoding of two possible future movements in the PPC (10). However, in these studies the animal is not free to choose which movement to perform but rather waits to receive an instructional cue. As a result, any reliable encoding of the plan that will actually be instructed, before receipt of the instructional cue, would violate causality and therefore indicate an error in the experimental design. In most tasks reported to date in which animals were free to select one of two options, those options were associated with differential reward probabilities or amounts, such that the animal's choices show a predictable bias (36–40). In these cases, LIP activity also shows a bias linked to the animal's choice bias. To test whether an activity bias occurs in the absence of differential rewards, we rewarded animals equally and at equal rates (100%) for each saccade, using the method of Huk and colleagues to ensure a more even distribution of saccade choices (41). We recorded from 15 cells in one animal (M1) using a two-target free-choice saccade paradigm with no reaching component. Even under these conditions, 13 of the 15 LIP cells showed the predicted behavior of coding the direction of the upcoming saccade, demonstrating that LIP's failure to code saccade direction in the bimanual-apart task is not a necessary consequence of presenting two saccade targets associated with equal reward probability and amount (Fig. S4).

A planned sequence of saccades does not explain why saccade direction is not encoded. It is possible that the similar activity we observe for saccades into and out of the RF on bimanual-apart reach trials is due to planning a sequence of saccades to the two targets. We address this possibility by examining those trials without a saccade sequence, that is, in which a saccade was made to only one of the two reach targets. Ten cells had at least two single-saccade trials in each of two matched conditions. There was no difference in bimanual-apart delay activity between E_{in} and E_{out} trials (E_{in} : median = four trials per cell, 23.69 ± 4.56 sp/s versus E_{out} : median = six trials per cell, 24.51 ± 4.59 sp/s, $P = 0.90$). The fact that we observe similar activity for saccades into and out of the RF regardless of whether the animal makes a single saccade or a sequence of two saccades is evidence that planning a sequence of saccades does not explain our results.

As an additional control, we noted that a sequence of saccades was sometimes performed in the free-choice task. Animals moved their eyes to the second target immediately after the first saccade in 7.1% of trials. The spatial and temporal patterns of saccade sequences in the free-choice task were similar to those in the bimanual-apart task. In each task, movements were made to the same two spatial locations with similar intersaccadic timing, averaging 221 ms for free-choice and 227 ms for bimanual-apart trials. The clear difference between E_{in} and E_{out} saccades in the free-choice task despite the presence of a saccade to each target confirms that planning a sequence of two saccades cannot explain the failure to encode the direction of the first saccade. Because the overall rate of saccade sequences was lower in the free-choice task than in the bimanual-apart task, we divided the free-choice cells into those with a high or low incidence of second saccades. We saw the same clear difference in both sets of

cells: 5.42 ± 2.63 sp/s in cells with high rates and 5.40 ± 2.12 sp/s in cells with low rates of second saccades ($P = 1.00$). Thus, we could detect no effect of executing a sequence of two saccades compared with a single saccade.

Subsets of LIP cells do not encode saccade direction. Hagan et al. (23) present evidence that only “saccade-preferring” LIP cells (that is, cells with significantly higher delay-period activity before a saccade compared with a reach plus saccade) are involved in eye–hand coordination. We grouped our LIP cells recorded during saccade-splitting behavior as saccade preferring ($n = 7$), reach plus saccade preferring ($n = 8$), or no preference ($n = 14$) and asked if saccade direction on bimanual-apart trials was coded by any of the groups. Saccade-preferring cells had overall higher firing rates, but the difference between E_{in} and E_{out} trials was similar for all three groups [differences of -2.91 ± 1.28 , -0.98 ± 2.28 , and -0.36 ± 1.33 sp/s, respectively; one-way ANOVA, $F(2, 26) = 0.65$, $P = 0.53$].

Alternative encodings of saccade direction in LIP. It is possible that delay activity encodes whether the initial saccade will accompany the contralateral or ipsilateral arm, independent of RF location. This was not the case. Across all 64 LIP cells, 49 provided data that could be used for this comparison. (For the other 15 cells, the eyes always accompanied the same arm.) Delay-period activity averaged 23.73 ± 2.71 sp/s when the initial saccade accompanied the contralateral arm and 23.84 ± 2.31 sp/s when the initial saccade accompanied the ipsilateral arm. These values are not significantly different from one another ($P = 0.78$).

Next, we considered a similar encoding, but relative to which arm movement in isolation evoked larger activity for the cell being studied, independent of RF. Of the 29 LIP cells that provided data that could be used for this comparison, seven preferred (i.e., showed significantly higher firing before a unimanual reach with) the contralateral compared with ipsilateral arm, and four preferred the ipsilateral arm. For these 11 cells, delay activity did not depend on whether the initial saccade accompanied the preferred or nonpreferred arm (24.81 ± 3.93 versus 25.15 ± 3.2 sp/s, respectively, $P = 0.86$).

Next, we considered not just saccade and reach directions but also RF location. For saccades that accompany the preferred arm, the firing rate might increase for movements into the RF (E_{in}) and decrease for movements out of the RF (E_{out}), with a reverse effect for saccades that accompany the nonpreferred arm (a decrease for E_{in} and an increase for E_{out}). As a result, E_{in} and E_{out} responses would, on average, be equal. To test for this, we ran a two-way repeated-measures ANOVA using all cells ($n = 64$), with the factors arm configuration (contralateral or ipsilateral arm into the RF) and saccade direction (eyes into or out of the RF), and looked at the interaction term. As expected from the analyses in Fig. 3, we found no main effect of saccade direction [E_{in} : 25.42 ± 2.27 sp/s vs. E_{out} : 22.26 ± 2.08 sp/s; $F(1, 131) = 1.80$, $P = 0.18$]. There was also no main effect of arm direction [A_{in} : 25.01 ± 2.05 sp/s vs. A_{out} : 22.24 ± 2.28 sp/s; $F(1, 131) = 1.15$, $P = 0.29$]. Critically, we found no interaction between the two main effects [$F(1, 131) = 0.71$, $P = 0.40$]. Repeating the ANOVAs but sorting based on whether the saccade accompanied the right versus the left arm (rather than based on whether the saccade was made into or out of the RF) again showed the same result. There was neither a main effect of saccade direction [$F(1, 131) = 1.80$, $P = 0.18$] nor an interaction between saccade direction and arm direction [two-way ANOVA, $F(1, 131) = 1.03$, $P = 0.31$]. Repeating the ANOVAs, this time weighting the contribution of each cell by the proportion of saccades made into or out of the RF during the recording, did not alter the results. There was no main effect of either saccade direction [RF sort: $F(1, 131) = 0.26$, $P = 0.61$; Arm sort: $F(1, 131) = 0.26$, $P = 0.61$] or arm direction [RF sort: $F(1, 131) = 1.20$, $P = 0.28$; Arm sort: $F(1, 131) = 0.26$, $P = 0.61$], and there was no interaction between saccade direction and arm direction

[two-way ANOVA; RF sort: $F(1, 131) = 0, P = 0.99$; Arm sort: $F(1, 131) = 0.50, P = 0.48$].

In summary, LIP delay-period responses on bimanual-apart trials did not encode saccade direction with respect to the RF, with respect to whether the eyes accompanied one or the other arm, or with respect to any combination of these factors.

We did find one effect of saccade direction on LIP activity. Although firing rates for E_{in} and E_{out} saccades were, on average, equal, there were small differences from cell to cell. We found that these small differences are weakly related to the fraction of E_{in} and E_{out} saccades that the animal chose to make while recording from that particular cell (Fig. S5). The correlation between the fraction of bimanual-apart trials in which the animal chooses to saccade into the RF is positively correlated with the mean firing rate on all bimanual-apart trials ($r = 0.38, P = 0.04$). This indicates that LIP does weakly represent spatial information related to saccade direction but not in a way that provides information about or may be causally related to the choice on any particular trial.

Effects of target blanking in LIP. In all the data presented so far, targets remained present on the screen throughout the delay period. The continuous presence of a visual stimulus in the RF could conceivably lead to a ceiling effect that masks any effect of saccade direction. To rule out this possibility, we recorded from 17 cells in one animal (M2) when the targets were blanked for 750 ms during the delay period. We computed the delay-period activity over the 500-ms interval beginning 250 ms after the stimuli disappeared and ending at the time the stimuli reappeared. There were at least two trials in each of two matched conditions for 8 of the 17 cells (Fig. S64). The population mean showed a drop in activity during the blanking period but still no coding of saccade direction (E_{in} : 21.09 ± 7.07 sp/s; E_{out} : 20.13 ± 6.89 sp/s; $P = 1.0$) (Fig. S6B). This was also true at the individual-cell level (all eight cells with $P > 0.05$) (Fig. S6C). The same was true for the presaccadic period. There was no difference in activity between the two conditions at either the population level (E_{in} : 38.38 ± 11.29 sp/s; E_{out} : 29.45 ± 10.24 sp/s; $P = 0.11$) (Fig. S6B) or the individual cell level (seven of eight cells with $P > 0.05$) (Fig. S6D).

PRR. Previous studies show that PRR cells preferentially encode upcoming arm movements compared with eye movements and that the majority of cells preferentially encode movements of the contralateral rather than the ipsilateral arm (4, 6, 7, 42). If PRR is involved in eye–hand coordination, then we might expect that PRR cells will reflect whether a saccade accompanies one arm or the other during a bimanual-apart reach or whether the saccade will be made into or out of the RF.

Fig. 4A shows the effect of saccade direction on the responses of an exemplary PRR cell in the bimanual-apart task. The pattern is similar to that observed in LIP cells. Delay activity was high for both E_{in} (solid magenta trace; $n = 25$ trials, 43.72 ± 7.02 sp/s) and E_{out} (dashed magenta trace; $n = 5$, 42.22 ± 15.91 sp/s; Wilcoxon rank-sum test, $P = 0.70$) conditions. The activity level increased in the 100 ms before the saccade for both bimanual conditions (E_{in} : solid magenta trace, 50.04 ± 6.06 sp/s; E_{out} : dashed magenta trace, 43.39 ± 14.96 sp/s; Wilcoxon rank-sum test, $P = 0.35$). In comparison, and as expected, activity was high for a unimanual reach plus coordinated saccade to a single target within the RF (solid black trace; $n = 30$ trials, 39.74 ± 6.55 and 38.32 ± 5.10 , sp/s for the delay and presaccadic periods, respectively) and low for a unimanual reach plus coordinated saccade outside the RF (dashed black trace; 1.56 ± 0.27 and 2.62 ± 0.56 sp/s for the delay and presaccadic periods, respectively). In both time intervals, both E_{in} and E_{out} bimanual responses were substantially greater than the unimanual out-of-RF response (Wilcoxon rank-sum tests, all $P < 0.001$), and neither bimanual response was significantly different from the unimanual in-RF response (Wilcoxon rank-sum tests, all $P > 0.40$).

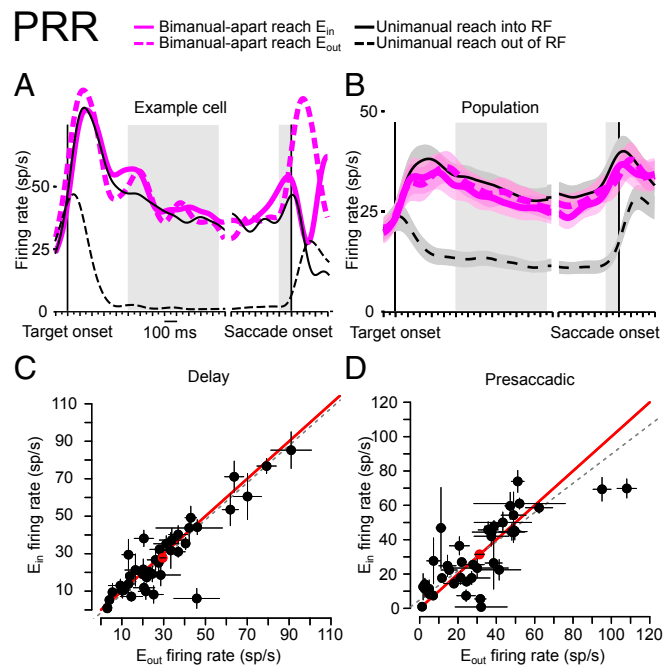


Fig. 4. PRR cells do not encode saccade direction during bimanual reaches. The format is as in Fig. 3. (A) Responses of an exemplary PRR cell. Responses did not vary as a function of saccade direction ($n = 25$ E_{in} trials, and $n = 5$ E_{out} trials; Wilcoxon rank-sum test: delay, $P = 0.70$; presaccadic, $P = 0.35$). (B) Population activity of PRR cells. All trials from each cell for which there were at least two trials of each saccade direction. Responses did not vary as a function of saccade direction ($n = 40$ cells; Wilcoxon signed rank-test: delay, $P = 0.23$; presaccadic, $P = 0.93$). (C and D) Scatterplots of the firing rates for E_{in} vs. E_{out} bimanual-apart reaches of the cells shown in B. Each point represents a single cell in the delay period (C) and presaccadic period (D). Error bars indicate the SEM. The unity line is in red. The dashed gray line is a type-II regression line.

Similar results held at the population level. Fig. 4B shows the average time course for 40 cells recorded when the animal chose to make at least two saccades into the RF (Fig. 2A, Upper) and at least two saccades out of the RF (Fig. 2A, Lower) on bimanual-apart trials with identical arm instructions. The median number of trials per condition was eight. Mean firing rates over the delay period were similar for E_{in} and E_{out} saccades (E_{in} : 27.76 ± 3.30 sp/s; E_{out} : 29.52 ± 3.32 sp/s; $P = 0.23$), and both were significantly greater than the mean unimanual response ($P = 0.03$ and 0.01 , respectively). This was also true for the 100 ms immediately before saccade onset (E_{in} : 31.24 ± 3.77 sp/s; E_{out} : 31.43 ± 3.31 sp/s; $P = 0.93$; both significantly greater than the mean unimanual response, $P = 0.01$ and 0.02 , respectively). At the individual-cell level, 3 of 40 cells showed a significant difference in firing rate between the E_{in} and E_{out} conditions during the delay period ($P < 0.05$) (Fig. 4C), a number not significantly different from that expected by chance (binomial test, $P = 0.45$). Eight cells showed a significant difference in firing rate between the E_{in} and E_{out} conditions immediately before the saccade (all $P < 0.05$), an effect greater than that expected by chance (binomial test, $P < 0.0007$) (Fig. 4D). However, the direction of the effect was not systematic, with five cells showing higher activity for E_{in} trials and three showing higher activity during E_{out} trials.

Activity peaked just at or immediately after the onset of saccades into the RF (solid traces) and then fell for both unimanual and bimanual-apart trials (Fig. 4B). For unimanual trials out of the RF, the pattern was very different, with a peak in activity 150 ms after saccade onset. In contrast, bimanual E_{out} trials resembled bimanual E_{in} trials, with a peak in activity at or just after saccade onset. This similarity was present at the individual-cell level, with 31 of 40 cells

showing no difference between E_{in} and E_{out} conditions in the postsaccadic (50- to 150-ms) period ($P < 0.05$) (Fig. S7).

Raising the criterion level for the minimum number of trials does not affect the results. With a criterion of four trials, delay activity differs by 0.58 ± 1.64 sp/s ($P = 0.73$, $n = 22$ cells), and presaccadic activity differs by 1.45 ± 2.34 sp/s ($P = 0.54$, $n = 22$ cells). With a criterion of six trials, delay activity differs by 0.49 ± 2.54 sp/s ($P = 0.85$, $n = 12$ cells), and presaccadic activity differs by 1.45 ± 2.34 sp/s ($P = 0.54$, $n = 12$ cells). A pooled analysis across all 89 cells yields similar results (delay: -4.09 ± 1.70 sp/s difference, $P = 0.17$; presaccade: -3.72 ± 1.69 sp/s, $P = 0.39$).

Alternative encodings of saccade direction in PRR: Interactions with arm configuration. The preceding analyses rule out a systematic effect of saccade direction on PRR activity during a bimanual movement, both in the delay period and even immediately before a saccade. We have less power to rule out an idiosyncratic (cell-specific) effect. By design, animals freely chose the direction in which they made their saccade on bimanual trials, and because we typically collected only 8–20 trials per cell for each of 10–40 trial types, most of our datasets contain fewer than eight trials for one configuration. (For example, with 20 right arm up/left arm down trials, if the animal chose to saccade up on 75% of trials and down on 25%, the trial counts would be 15 and 5. We feared that doubling or quadrupling the number of trials might lead to more stereotyped behavior.) As a result, we do not have the power to rule out significant effects of coordinated saccade direction at the individual cell level. In LIP, there is a very strong prior for higher activity across most cells when the coordinated saccade is made into the RF, independent of what the arms do. In PRR, this prior is much weaker. However, we can test other systematic effects that involve interactions between arm and eye movements.

We previously showed that PRR activity reflects arm configuration during a bimanual-apart reach using an entirely different dataset than that used here (6). Activity was greater when the contralateral arm moved into the RF and the ipsilateral arm moved out of the RF, compared with when the contralateral arm moved out and the ipsilateral arm moved in. We therefore asked if there might be an interaction between arm configuration and saccade direction in PRR. We ran a two-way repeated-measures ANOVA using all cells ($n = 89$), with the factors arm configuration (contralateral or ipsilateral arm into the RF) and saccade direction (into or out of the RF). There was no main effect of either arm configuration [A_{in} : 32.26 ± 2.19 sp/s vs. A_{out} : 25.02 ± 2.32 sp/s; $F(1, 204) = 2.75$, $P = 0.1$] or saccade direction [E_{in} : 28.51 ± 2.11 sp/s vs. E_{out} : 29.34 ± 2.44 sp/s; $F(1, 204) = 0.24$, $P = 0.62$], as is consistent with the analysis in Fig. 4. Importantly, arm configuration did not interact with saccade direction [two-way ANOVA, $F(1, 204) = 0.01$, $P = 0.92$]. Repeating the ANOVA but sorting based on whether the saccade was coordinated with the right versus left arm (rather than based on whether the saccade was made into or out of the RF) again showed neither a main effect of saccade direction [$F(1, 204) = 0.24$, $P = 0.62$] nor an interaction effect [$F(1, 204) = 0.39$, $P = 0.53$]. Repeating the ANOVAs, this time weighting the contribution of each cell by the proportion of saccades made into or out of the RF during the recording, did not alter the results. There was a main effect of arm direction [sort based on saccades into versus out of the RF: $F(1, 204) = 19.44$, $P = 0.000017$; sort based on saccades accompanying the left or right arm: $F(1, 204) = 24.24$, $P = 0.0000017$]. There was no main effect of saccade direction [RF sort: $F(1, 204) = 0.77$, $P = 0.38$; arm sort: $F(1, 204) = 0.77$, $P = 0.38$]. There was no interaction when the sort was based on RF [two-way ANOVA $F(1, 204) = 0.65$, $P = 0.42$]. There was an interaction when the sort was based on the arm [$F(1, 204) = 4.23$, $P = 0.04$, which would not be significant if corrected for multiple comparisons (eight ANOVAs)].

Although firing rates for E_{in} and E_{out} saccades were, on average, equal, there were small differences from cell to cell. We tested whether these small differences might be related to the fraction of E_{in} and E_{out} saccades that the animal chose to make while recording from a particular cell. Unlike LIP, the correlation between the fraction of bimanual-apart trials in which the animal chooses to saccade into the RF while recording from a given cell is not significantly correlated with the mean firing rate across all bimanual-apart trials from that cell ($r = 0.17$, $P = 0.30$). This confirms that PRR does not represent spatial information related to saccade direction.

A planned sequence of saccades does not explain why saccade direction is not encoded. Similar to the results in LIP, planning a sequence of saccades did not explain the loss of directional coding in PRR. Even when considering only single-saccade trials, there was still no difference between E_{in} and E_{out} trials (18 cells for which there were at least two single-saccade trials in each of two matched conditions; E_{in} : median = 10.5 trials per cell, 25.41 ± 5.01 sp/s; E_{out} : median = 9 trials per cell, 27.43 ± 5.21 sp/s, $P = 0.77$).

No functional differences across cytoarchitectonic areas. The population of 40 PRR cells recorded when the animal chose to make at least two saccades into and at least two saccades out of the RF comprised equal numbers of cells from MIP ($n = 14$), the parietal-occipital area (PO) ($n = 13$), and the lateral occipital-parietal area (LOP) ($n = 13$) (proportion test, $P = 0.96$). Delay-period activity on E_{in} versus E_{out} trials was similar within each area (MIP: 24.69 ± 4.13 versus 24.23 ± 5.03 sp/s, $P = 0.71$; PO: 24.35 ± 6.53 versus 29.15 ± 5.72 sp/s, $P = 0.19$; LOP: 34.15 ± 6.46 vs. 35.20 ± 6.88 sp/s, $P = 0.72$). This was also true for the presaccadic period (MIP: 29.96 ± 5.62 vs. 27.85 ± 8.29 sp/s, $P = 0.81$; PO: 25.82 ± 5.00 vs. 26.70 ± 4.69 sp/s, $P = 0.86$; LOP: 41.95 ± 6.34 vs. 39.51 ± 6.80 sp/s, $P = 0.52$) and for the postsaccadic period (MIP: 33.48 ± 6.89 vs. 29.64 ± 8.76 sp/s, $P = 0.35$; PO: 34.46 ± 6.92 vs. 32.44 ± 5.78 sp/s, $P = 0.65$; LOP: 41.67 ± 7.79 vs. 48.59 ± 8.10 sp/s, $P = 0.24$).

Discussion

We exploited the natural variability in saccade direction that occurs when monkeys reach simultaneously to two different targets, one with each arm, to study eye–hand (saccade–reach) coordination. If cells help coordinate saccades with reaches, then their activity should depend on the pattern of eye–hand coordination. In our task, eye movements were unconstrained once the cue to reach was given. Animals naturally coordinated their eyes to the target of one arm or the other. In every previous study involving eye movements, most LIP cells increased their activity when a saccade was directed into the RF. Surprisingly, LIP cells showed no systematic effect of saccade direction when the animals reached to two separate targets (bimanual-apart task), even in the 100 ms immediately before saccade onset. Cells in PRR also failed to show a systematic effect of saccade direction. Thus, neither PRR nor LIP systematically encodes eye–hand coordination during a bimanual reach task.

In the laboratory, eye–hand coordination is typically studied using unimanual (one-arm) reaches to a target. In the absence of special training, a coordinated saccade accompanies the reach on most trials, making it difficult to ascertain whether any particular neural activity is related to coordination. Training the subject to spatially or temporally dissociate the saccade from the reach makes this possible but at the same time risks bypassing or altering the very circuits that are intended as the focus of the study. We instead dissociated eye and arm movements by asking animals to simultaneously reach with each arm to a different target. The animal naturally saccades first to one target and then to the other (Fig. 2B). This bimanual task approximates the complexity of eye–arm interactions in more natural settings, where fixations tend to fall on task-relevant objects and not elsewhere (43). It is therefore not surprising that we observe sequential saccadic eye movements to the two reach targets. Each saccade is spatially congruent with

just one of the two reaches. By dissociating the first saccade from one of the two reaches, we can more easily ask which brain area(s) show activity related to eye–hand coordination.

The finding that PRR shows no effect of whether a saccade is coordinated with the contralateral or ipsilateral arm is consistent with some, but not all, previous studies. Although evidence from single-unit recording and brain damage has implicated the PPC in eye–hand coordination (2, 24, 44, 45), unilateral inactivation of PRR has shown discrepant results. In two studies, inactivation of PRR failed to affect the temporal correlation between eye and hand reaction times, arguing against a causal role in eye–hand coordination (16, 20). A third study reported decreased temporal correlation between eye and hand reaction times, although the effect was only observed for a single contralateral target location (21). Several important differences, including the specific anatomical location of the injections, task design, and injection volumes, may explain the discrepancies among studies (discussed in ref. 21).

LIP results, in contrast, are completely unexpected. LIP activity has been interpreted as encoding the goal of an upcoming saccade (“saccade intention”), a signal that drives the focus of spatial attention (“priority map”), a high-level cognitive signal, or some combination of these signals (3, 46–51). All these models predict differential activity for saccades made into or out of a cell’s RF. Indeed, while many factors modulate LIP firing, LIP cells consistently encode the direction of an upcoming saccade across a broad range of paradigms, even when other factors are simultaneously encoded (37, 52). It is therefore remarkable that the large majority of LIP cells do not encode saccade direction in the bimanual-apart paradigm until after the saccade has been initiated (Fig. 3 and Fig. S3). This is not a consequence of choosing between two equally valuable saccade targets, since, in the absence of bimanual-apart arm movements, there is clear coding for the first saccade (Fig. S4). When arm movements are present, however, the effect of saccade direction on LIP (and PRR) activity is abolished in almost all cells. We cannot rule out the possibility that the small numbers of cells that still show significant effects could play an outsized role in function, although the fact that some of these cells show greater firing for out than for in suggests that they may instead be statistical anomalies.

There are other possible explanations for our results that would preserve a role for the PPC in eye–hand coordination. Since activity is high for saccades both in and out of the RF in our task, it is possible that activity plays a permissive rather than instructive role. Rather than directly driving a coordinated eye movement, PPC activity might provide a downstream area with one or more options as to where to direct the eyes, allowing the downstream area to make the final decision. In other words, PPC activity might be necessary but not sufficient for eye–hand coordination. The distinction might not be obvious in a study using only a single prospective target. Our results are consistent with evidence from the neuropsychological literature implicating the PPC in visually guided reaching (2, 53) and bimanual coordination (54). Our data clearly show a role for the PPC in the arm component of visually guided reaching. Any PPC lesion that compromises visually guided reaching might thereby indirectly perturb measures of bimanual coordination. It is difficult to isolate a specific effect on bimanual coordination that is not secondary to an effect of visually guided reaching. Similarly, a number of studies have proposed the PPC as a likely region for eye–hand coordination but have not provided direct evidence, for related reasons (24, 45, 55, 56).

Another possibility is that there are cell-specific contributions to eye–hand coordination in the bimanual task that cancel out at the population level. This seems unlikely in LIP, where in almost all published studies there is a systematic effect of saccades made into compared with out of the RF. In PRR, the case is less clear. PRR

cells code movements of the contralateral arm in a systematic fashion, while particular patterns of bimanual coordination appear to have idiosyncratic effects on activity that cancel out at the population level (6, 7). Finally, one can imagine many alternative neuronal mechanisms, other than mean firing rate, that might influence eye–hand coordination. Recent evidence suggests that spike local field potential coherence in the beta-frequency band is important for eye–hand coordination (23–25). Alternatively, coordination may arise from population activity functioning as part of a dynamical system (e.g., ref. 57). Any of these mechanisms may depend on interactions across multiple cortical and/or subcortical areas instead of on local activity alone.

The lack of a systematic LIP response cannot be explained by an attentional account. For attention to explain the insensitivity to the direction of the saccade on bimanual-apart trials, one would have to argue that animals split their attention equally to the two possible targets. However, attention and saccade direction are linked. Even if attention and saccade direction are completely dissociated during the delay period, it is known that attention will shift to an upcoming saccade endpoint shortly before the start of a saccade (58–60). Therefore, we should see that LIP firing reflects saccade direction at this time. This has been seen in related experiments (61, 62) as well as in our free-choice saccade control task (Fig. S4), but we do not see this during the bimanual-apart trials (Fig. 3). The lack of directional coding immediately before saccade onset is as problematic for an attentional interpretation of LIP activity as it is for a motor interpretation. Importantly, even if one accepts the attentional interpretation of LIP activity, the conclusion from our data that LIP does not play a causal role in eye–hand coordination still holds.

Our findings of similar activity on bimanual-apart trials with eye movements either into or out of the RF are consistent with the proposition that competing movement goals may be represented in parallel in some cortical areas, the “affordance competition” hypothesis (63). The parallel representations are also consistent with LIP playing a permissive role in coordinating the saccade with either of the two arm movements. However, the fact that activity is similar right up until the time of the saccade means that LIP cannot play a causal role in directing the saccade or, more generally, in mediating any aspect of eye–hand coordination that depends on whether the eyes move with the left or right arm. (As described in *Results*, this argument applies to population-level activity; we cannot rule out a role of small cell-specific differences in firing.) The same argument applies to PRR: The fact that activity is similar when the eyes move with either the left or right arm is consistent with PRR coding both movement plans but rules out a causal role directing the saccade or coordinating the saccade with the reach, at least at the population level.

In this regard, it is worth noting that in most studies of the affordance competition hypothesis representations of two plans are indistinguishable at times before the animal receives an instruction to choose one or the other plan (64, 65). Once such an instruction is received, the two representations diverge. In free-choice tasks, this divergence occurs hundreds of milliseconds before movement onset in PRR (66). In one study (65), two potential movement plans were introduced at the start of each trial, and then, some time later, a disambiguating cue was given in ~75% of trials that effectively instructed one plan or the other. Before the delivery of this disambiguating cue, both plans are equally represented at the population level. This was true in both the 75% of trials in which the cue was delivered and in the 25% of trials in which it was not delivered. In the latter trials, the authors do not report whether activity evolves to reflect the animal’s choice once it knows that it is indeed free to choose but before the onset of movement. This is certainly possible, especially given that animals have 700–1,000 ms to perform the movement once the go cue is given. Thus, our data are consistent

with the affordance competition hypothesis but not with population-level LIP or PRR activity playing more than a permissive role in coordinating a saccade with either one or the other limb in a bimanual-apart reach task.

The affordance competition hypothesis has been investigated primarily using plans to reach. We used a free-choice saccade task to determine whether choosing to saccade to either of two targets in the absence of any arm movements would result in equal representation of the two possible movement plans. Under these circumstances, and in contrast to the main (bimanual-apart) task, the free-choice saccade task showed greater activity for E_{in} compared with E_{out} trials during the delay up through the time of the saccade (Fig. S4), as is consistent with previous studies of free-choice saccades in LIP (61, 62). It is possible that in the bimanual-apart task the animal does not commit to one saccade or the other until the moment of saccade execution. Once again, however, the absence of a difference in firing before saccade onset indicates that LIP does not play a causal role in making that decision.

We conclude that, in the special case of a bimanual movement to two different targets, LIP signals the possible saccade goal locations but does not actually select the saccade that is performed (although it may contribute to an overall bias) (Fig. S5). We do not wholly rule out some role for LIP in eye–hand coordination, since an LIP lesion slows coordinated saccades during a unimanual reach, suggesting that LIP plays a permissive role in eye–hand coordination for unimanual reaches (23). Furthermore, the fact that neither LIP nor PRR codes saccade direction in a bimanual reach does not preclude the exchange of information between these areas assisting in spatial targeting in these tasks. More experiments are required to determine how LIP's role differs during unimanual and bimanual tasks and whether and how information might be shared between these regions.

Materials and Methods

Subjects. All procedures conformed to the *Guide for the Care and Use of Laboratory Animals* (67) and were approved by the Washington University Institutional Animal Care and Use Committee. Two male rhesus macaques (*Macaca mulatta*) (M1 and M2) participated in the study. A similar task design was used for two previously published studies (6, 33), but there is no overlap in the data used in those studies and this study.

Delayed Movement Tasks.

Visually guided tasks. The task design and the movement conditions are shown in Fig. 1A. Details of the experimental apparatus are provided in *SI Methods*. The main task was a reach with both arms to one or two targets. Animals first fixated ($\pm 3^\circ$) on a circular white stimulus ($1.5^\circ \times 1.5^\circ$) centered on the screen in front of them. Left and right hands touched home pads situated at waist height and 20 cm forward of each shoulder. After 500 ms, red and green peripheral targets ($5^\circ \times 5^\circ$) appeared on the screen and remained present for a 1,250- to 1,750-ms delay period. The two targets were always diametrically opposite to one another across the central fixation point at one of eight equally spaced locations. At the end of the delay period, the central target was extinguished, cueing the animal to reach with the right arm to the red target and with the left arm to the green target. Interleaved with this principle bimanual-apart task were four other trial types. Unimanual left or right arm reaches were instructed with a single green or red peripheral target, respectively. Reaches with both arms to a single target (bimanual-together) were instructed with a blue target, and saccade-only trials (no reach) were instructed with a white target. Throughout saccade and unimanual reach trials, the hand(s) that were not instructed to move were required to remain on the home pad(s). On unimanual reach trials, eye movements to the target of the reach were required. On bimanual reach trials, eye movements were unconstrained once the animals were cued to initiate the movement, and the left and right hands were required to hit their targets within 500 ms of one another. In fact, bimanual movements were temporally coordinated with one another, with nearly synchronous reach initiation (Pearson r coefficient; M1: together, 0.97; apart, 0.96; M2: together, 0.97, apart, 0.95) and completion times (M1: together, 0.96; apart, 0.93; M2: together, 0.97, apart, 0.85). In 80% of trials, the left and right hands reached their targets within 77 ms (bimanual-together) or 137 ms

(bimanual-apart) of one another, despite differences in distance from the home pads to the targets. Spatial tolerances were $\pm 3^\circ$ for reaches and $\pm 2^\circ$ for saccades. For all trial types, the final arm configuration was held for 250 ms. When an error (i.e., a failure to achieve or maintain the required eye or hand positions) occurred, the trial was aborted, and a short (1,500-ms) time-out ensued. Aborted trials were excluded from further analyses. Successful trials were rewarded with a drop of water or juice.

Target-blanking task. For one animal (M2), additional data were collected in trials in which the target disappeared for a short duration ($n = 19$ cells). The events and timing were the same as in the visually guided tasks except that the visual target(s) disappeared 500 ms after onset and remained off for 750 ms. The cue to move was given 0–500 ms after target reappearance.

Free-choice saccade task. For one animal (M1), additional data were collected in saccade trials in which two white targets appeared instead of one ($n = 15$ cells). All aspects of the task were identical to the one-target saccade task, except that the target eccentricity and fixation positions were varied such that animals chose each target between 28% and 72% of the time except for one cell in which one target was chosen 89% of the time. The animal was rewarded equally for a saccade to either target.

Electrophysiological Recordings. Single-unit recordings were made from all four hemispheres in two adult male rhesus monkeys. We identified boundaries for LIP and PRR based primarily on physiological criteria. See *SI Methods* for additional recording details.

Quantification and Statistical Analysis.

Measurement of cell activity. We computed the spike rate in the delay (500–1,250 ms after target onset), presaccadic (100 ms before saccade onset), and postsaccadic (50–150 ms after saccade onset) epochs. To show firing rate as a function of time, we computed reciprocal interspike intervals with 1-ms resolution, smoothed the data using a low-pass filter with a -3 dB point at 8 Hz (mathematically identical to convolving with a Gaussian with an SD of 17 ms) and then averaged the smoothed values across trials.

Movement direction. The present report focuses on saccade direction during bimanual-apart reaches, that is, trials in which animals were instructed to reach simultaneously with the left arm to one target and with the right arm to a second target. One target was in the RF of the cell being recorded, and the other was outside the RF. Trials were sorted based on whether the initial saccade was directed to the target inside or outside the RF. We refer to these as “eye-in” (E_{in}) and “eye-out” (E_{out}) trials, respectively. This report focuses on activity before the first saccade. In a secondary analysis, trials were sorted based on whether the contralateral arm moved to the target inside or outside the RF. We refer to these as “arm-in” (A_{in}) and “arm-out” (A_{out}) trials, respectively. Thus, there were four possible trial types: $E_{in}A_{in}$, $E_{out}A_{in}$, $E_{in}A_{out}$, and $E_{out}A_{out}$. Reach directions were instructed, such that we obtained equal numbers of A_{in} and A_{out} trials for each cell. Saccade direction was freely chosen by the animal on each trial, so the relative numbers of E_{in} and E_{out} trials varied from cell to cell. For example, if the animal always chose to saccade to the right in a particular recording session, we might obtain only $E_{out}A_{in}$ and $E_{out}A_{out}$ trials for that cell. We could obtain two, three, or four different bimanual-apart trial types for a given cell. In most cases, we restricted our analyses to cells for which we obtained at least two $E_{in}A_{in}$ and two $E_{out}A_{in}$ trials or at least two $E_{in}A_{out}$ and two $E_{out}A_{out}$ trials (just under half of all cells: 40 in PRR and 29 in LIP). The number of trials per cell varied, with means of 9.8 and 9.5 trials, respectively, for the main task in LIP (range, 2–34 and 2–26 trials for E_{in} and E_{out} trials) and with means of 9.6 and 11.5 trials, respectively (range, 2–56 and 2–34 trials) for the main task in PRR. For the target-blanking task in LIP, means were 5.1 and 8.6 trials, respectively (range, 2–10 and 3–12 trials), and for the two-target free-choice saccade task in LIP the means were 61.7 and 54.7 trials, respectively (range, 3–121 and 3–168 trials).

Statistics. For statistical analysis, we used nonparametric tests appropriate for data that are not necessarily normally distributed. All tests were Wilcoxon signed-rank tests (paired comparisons) except where Wilcoxon rank-sum tests (pooled comparisons) are noted. All tests were two-sided except where noted. To evaluate interactions, we used repeated-measures ANOVA, which is robust to modest violation of normality with sufficient sample size (68, 69). The criterion for all tests was $\alpha = 0.05$. Firing rates are reported as the mean \pm SEM.

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