Beyond the labeled line: variation in visual reference frames from intraparietal cortex to frontal eye fields and the superior colliculus

ABBREVIATED TITLE

Visual reference frames vary in FEF, LIP/MIP, SC

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ABSTRACT

We accurately perceive the locations of visual stimuli despite the fact that our eyes move roughly three times per second. The neural computations that underlie this ability require incorporation of eye position and retinal information. Here, we assessed how single neurons do this in three interconnected structures: the frontal eye fields (FEF), the parietal cortex (LIP/MIP), and the superior colliculus (SC). Head-restrained monkeys performed visually-guided saccades from different initial eye fixations. By comparing visual responses across eye positions, we quantified whether the receptive fields were in eye-centered, head-centered, or hybrid coordinates. As previously shown, most LIP/MIP neurons encoded visual signals in hybrid coordinates, i.e. their receptive fields shifted to novel positions on the retina for each eye position, and these locations were not clearly

related to each other in either eye- or head-centered coordinates. In contrast, most SC neurons exhibited receptive fields that had a stable position in eye-centered coordinates. In the FEF, visual signals were intermediate between those patterns: around 60% of the recorded neurons encoded targets in eye-centered coordinates, whereas the remainder showed changes in receptive field location, boundaries, or responsiveness that rendered the response patterns hybrid or occasionally head-centered. These results suggest that FEF may act as a transitional step in an evolution of coordinate systems between LIP/MIP and the SC. The persistence across cortical areas of hybrid representations that do not provide unequivocal location labels in a consistent reference frame has implications for how these representations must be read out.

SIGNIFICANCE STATEMENT

How we perceive the world as stable using mobile retinas is poorly understood. We compared neuronal responses in three regions important for visuomotor behavior: frontal eye fields (FEF), intraparietal cortex (LIP/MIP), and superior colliculus (SC). The first two (cortical) regions used a hybrid code that includes information about not only the visual scene but also the eye position, akin to the “camera angle” viewing the scene. Hybrid coding was ubiquitous in LIP/MIP, evident but less common in FEF, and negligible in the SC, where eye-centered coding dominated. Neurons exhibiting hybrid coding cannot form the labeled-line code for stimulus location usually assumed in these cortical areas. However, a labeled-line is available at the output stage from these areas, the eye-central SC.

KEYWORDS:

reference frame, coordinate system, coordinate transformation, visual, saccade, frontal eye field (FEF), superior colliculus (SC), lateral intraparietal cortex (LIP), medial intraparietal cortex (MIP), labeled line

INTRODUCTION

The perceived locations of visual stimuli are derived from a combination of the location of retinal activation and information about the direction of eye gaze. How these signals are combined to synthesize a representation of visual space as the eyes move is unknown. The process is computationally complex because any site on the retina could correspond to any given site in the visual scene, but only the correct correspondence for a particular eye gaze position is operative at any given time. Coordinate transformations are therefore both flexible and precise, and it has been suggested that they unfold as a gradual process across multiple brain regions.

Which visual areas are truly retinotopic, or eye-centered, and which employ higher-order representations incorporating information about eye movements is uncertain. The retina is thought to provide the brain with an eye-centered map of the locations of visual stimuli. But after that, the recurrent interconnectivity of the brain in principle permits adjustment of reference frame. Several studies have indicated effects of eye position or movement on visual responses as early as the lateral geniculate nucleus (Lal and Friedlander, 1989, 1990a, b). In V1, some studies have found evidence that eye position modifies visual signals (Trotter and Celebrini, 1999) and some have not (Motter and Poggio, 1990; Gur and Snodderly, 1997). Later visual structures exhibit more extensive sensitivity to eye position/movements (e.g. Squatrito and Maioli, 1996; Bremmer et al., 1997; Bremmer, 2000).

We focus here on a quantitative comparison of the reference frames employed in three interconnected brain regions involved in guiding saccadic eye movements to visual targets (Figure 1): the lateral and medial banks of the intraparietal sulcus (LIP/MIP), the

superior colliculus (SC), and the frontal eye fields (FEF) (e.g. Wurtz and Goldberg, 1971; Sparks et al., 1976; Bruce and Goldberg, 1985a; Huerta et al., 1986; Lee et al., 1988; Stanton et al., 1988; Waitzman et al., 1988; Schall, 1991; Schall and Hanes, 1993; Freedman and Sparks, 1997; Wurtz et al., 2001; Scudder et al., 2002; Bisley and Goldberg, 2003; Steenrod et al., 2013). Electrical stimulation in the SC and FEF evokes short latency saccades at low thresholds (Robinson and Fuchs, 1969; Robinson, 1972; Schiller and Stryker, 1972; Bruce et al., 1985). Stimulation in parietal cortex can also evoke saccades (Thier and Andersen, 1996, 1998). Lesions of both the SC and FEF together eliminate saccades whereas lesions of parietal cortex can produce hemineglect (Bisiach and Luzzatti, 1978; Schiller et al., 1980; Schiller et al., 1987; Sommer and Tehovnik, 1997; Dias and Segraves, 1999).

Parietal cortex was one of the first brain regions in which eye movements and visual signals were shown to interact (Andersen et al., 1993). These response patterns were initially characterized as “gain fields”, in which receptive fields were stable in eye-centered location but exhibited a response modulation with different eye positions (Andersen et al., 1985). Subsequent studies involving complete sampling of receptive field positions as the eyes moved suggested that receptive fields could also adopt new positions on the retina at different eye positions (Mullette-Gillman et al., 2005, 2009). These changes in receptive field position produced a code that varies across neurons and ranges from predominantly eye-centered to predominantly head-centered, with most neurons exhibiting “hybrid” response patterns. In contrast, the SC, while exhibiting gain fields (Van Opstal et al., 1995), employs a predominantly eye-centered code when tested and analyzed the same way as LIP/MIP (Klier et al., 2001; Lee and Groh, 2012).

Considerable interest has focused recently on eye position gain fields in the FEF (Cassanello and Ferrera, 2007) and on how receptive fields in the FEF change transiently at the time of the eye movement (Sommer and Wurtz, 2006; Zirnsak and Moore, 2014)). However, a quantitative assessment of the reference frame during steady fixation has not, to our knowledge, been conducted. Here we report that the reference frame of visual signals in the FEF is intermediate between the SC and LIP/MIP. The results support the view that reference frames evolve along brain pathways involved in controlling visually-guided behavior, becoming a plausible labeled line for eye-centered stimulus location only at the level of the SC.

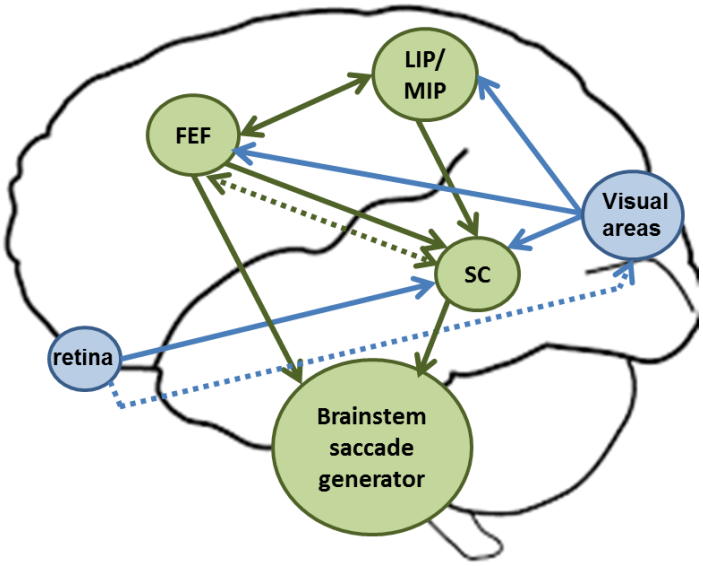

Figure1: Schematic of the connections between the areas LIP/MIP, SC and FEF, their visual inputs and their projections to the brainstem saccade generator. *The LIP/MIP and FEF are highly interconnected and send excitatory projections to the intermediate and deep layers of the SC (continuous arrows indicate direct projections). The FEF also sends inhibitory indirect projections to the SC through the caudate and the*

substanta nigra pars reticulata (dotted arrows indicate indirect projections). Both the SC and the FEF directly project to the various areas of the brainstem saccade generator system. The LIP/MIP and the FEF receive visual inputs from extrastriate visual areas. The SC receives visual inputs mainly in its superficial layer from the primary and secondary visual cortices and the FEF, and also directly from the retina. (for reviews, see Sparks and Hartwich-Young, 1989; Blatt et al., 1990; Schall et al., 1995)

MATERIALS AND METHODS

The task and recording conditions for the FEF dataset have been explained previously (Caruso et al., 2016). We briefly report them here. All experimental procedures conformed to NIH guidelines (2011) and were approved by the Institutional Animal Care and Use Committee of Duke University. Two adult rhesus monkeys (*Macaca mulatta*) were implanted with a head holder to immobilize the head, a scleral search coil to track eye movements and a recording cylinder over the left or right FEF. Similar procedures were used to prepare for recordings in LIP/MIP and SC, as reported previously (Lee and Groh, 2014) (Lee and Groh, 2012) (Mullette-Gillman et al., 2005, 2009)

All data were recorded while the monkeys performed visually or auditory guided saccades, randomly interleaved, in an overlap saccade paradigm. Only visual trials were analyzed in this study. In each trial, a target was presented while the monkey fixated a visual fixation stimulus (Figure 2A, B; all visual stimuli were produced by green light emitting diodes, LEDs). The monkey withheld the saccade for 600-900 ms until the offset of the fixation target, permitting the dissociation of sensory-related activity from

motor-related activity. The targets were located in front of the monkeys at 0° elevation and between -24° and +24° horizontally (nine locations separated by 6°, figure 2A). In each session, all saccades started from three initial fixation locations at -12°, 0°, +12° along the horizontal direction and at an elevation chosen to best sample the receptive field of the neuron under study.

The behavioral paradigm, the acquisition of eye trajectory and the recordings of single cell activity were controlled using the Beethoven program (Ryklin Software). Eye gaze was sampled at 500Hz. Single neuron extracellular activity was acquired using a Plexon system (Sort Client software, Plexon) through tungsten micro-electrodes (FHC, 0.7 to 2.5 MOhm at 1 kHz).

Analysis

All analyses were conducted with custom-made routines in Matlab (the MathWorks Inc.). Only correct trials were included.

Spatial selectivity analysis: This analysis has been described in detail in (Caruso et al., 2016), (Mullette-Gillman et al., 2005) and (Lee and Groh, 2012). Briefly, we defined a **baseline period**, comprising the 0-500 ms of fixation before the target onset and a **sensory window** as the period 0-500 ms after target onset. The sensory window captured both the transient and sustained responses to visual targets (We selected the **motor window** differently in different areas, to better capture the saccade related burst, which has different temporal characteristics across regions. The motor window was defined to start before the saccade onset (20ms before saccade onset for the SC data, 50ms before for the FEF data and 150ms before for the LIP data) and to end at saccade offset. Saccade onset and offset were defined as the moment, at 2 ms resolution, that the

instantaneous speed of the eye movement exceeded or dropped below a threshold of 25°/s. (In addition to these fixed analysis windows, we also analyzed sliding 100 ms windows throughout the interval from target onset to the saccade, detailed below at the end of this section).

Neurons were considered **responsive** in the sensory/motor intervals, if a two-tailed t-test between their baseline activity and relevant response period was significantly different with $p<0.05$. Spatial selectivity of responses (in the sensory or motor period) was assessed in both head- and eye-centered reference frames, using two two-way ANOVAS. Each ANOVA involved the three levels of initial eye position (-12°, 0°, +12°) as well as five levels of target location (-12° to +12° in 6° increments), defined in head-centered coordinates for the first ANOVA and in eye-centered coordinates for the second ANOVA. Cells were classified as **spatially selective** if either of the two ANOVAs yielded a significant main effect for target location, or a significant interaction between the target and fixation locations (Mullette-Gillman et al., 2005, 2009; Lee and Groh, 2012, 2014). In all tests, statistical significance was defined as p value < 0.05. To be consistent with our previous analyses and because these tests were used for inclusion criteria rather than hypothesis testing, we did not apply Bonferroni correction.

Reference frame analysis: To distinguish eye-centered and head-centered reference frames, we quantified the degree of alignment between eye-centered and head-centered tuning curves obtained from trials with initial eye positions at -12º, 0 º, +12º along the horizontal axis. This analysis was applied to single cells during different time windows throughout the trials. In particular, for each time window considered, we constructed the three response tuning curves for the three fixation locations with target

locations defined in head- or eye-centered coordinates (schematized in figure 2) and quantified their relative shift with an index akin to an average correlation coefficient (equation1, (Mullette-Gillman et al., 2005)). We call it **reference frame index** and for each response we calculate two indexes, in head-centered and in eye-centered coordinates, according to the formula:

$$R = \sum_i \frac{1}{2}\left(\frac{(\overrightarrow{r_{L,i}}-\bar{r})\cdot(\overrightarrow{r_{C,i}}-\bar{r})}{|\overrightarrow{r_{L,i}}-\bar{r}|\cdot|\overrightarrow{r_{C,i}}-\bar{r}|} + \frac{(\overrightarrow{r_{R,i}}-\bar{r})\cdot(\overrightarrow{r_{C,i}}-\bar{r})}{|\overrightarrow{r_{R,i}}-\bar{r}|\cdot|\overrightarrow{r_{C,i}}-\bar{r}|}\right) \quad (1)$$

where $r_{L,i}$, $r_{C,i}$, and $r_{R,i}$ are the vectors of average responses of the neuron to a target at location *i* when the monkey's eyes were fixated at the left (L), right (R), or center (C). Only the target locations that were present for all three fixation positions in both head- and eye-centered frames of reference were included (5 locations: -12, -6, 0, 6, and 12°). The reference frame index is primarily sensitive to the relative translation of the three tuning curves and does not measure possible gain differences between them. Its values range from -1 to 1: 1 indicates perfect alignment, 0 indicates no alignment, -1 indicates perfect negative correlation (Mullette-Gillman et al., 2005; Porter and Groh, 2006; Mullette-Gillman et al., 2009; Lee and Groh, 2012, 2014). We calculated the 95% confidence intervals of the index with a bootstrap analysis (1000 iterations of 80% of data for each target/fixation combination). Each set of responses was classified based on the quantitative comparison between the eye- and head-centered indices as: 1) **eye-centered**, if the 95% confidence interval of eye-centered reference index was positive and larger than the 95% confidence interval of head-centered reference index; 2) **head-centered**, if the opposite pattern was found; 3) **hybrid-partial shift**, if the 95% confidence intervals

of the eye-and head-centered indices overlapped with each other and at least one did not overlap with zero; **4) hybrid-complex**, if the 95% confidence intervals of the eye-and head-centered indices overlapped with each other and with zero (Figure 4). These latter two categories were combined in our previous reference frame analyses of activity patterns in the SC and LIP/MIP. (Mullette-Gillman et al., 2005, 2009) (Lee and Groh, 2012). Here, we consider them both separately and together as appropriate to provide a more comprehensive comparison of the reference frames across regions. We conducted the reference frame analysis for each cell during the sensory and motor periods (figure 4A,B and 5A,B) and across time in 100ms windows sliding with steps of 50ms from the target onset and from saccade onset (figure 4C,D, and 5C,D).

LIP/MIP and SC datasets

Figure 5 includes data from area LIP/MIP and SC that we have previously collected and described ((Mullette-Gillman et al., 2005, 2009; Lee and Groh, 2012, 2014)) .The tasks and recording techniques were the same as in the present study, but each dataset was recorded from different monkeys (two monkeys per brain area). The SC dataset consists of a total of 179 single cells recorded in the left and right SC of two monkeys. The LIP/MIP dataset consists of a total of 275 single cells recorded in the left and right LIP/MIP of two monkeys. For these two additional datasets, responsiveness, spatial selectivity and reference frame were assessed the same way as for the FEF data set. Some of the data in figure 5 were reanalyzed in different time windows than the original studies, to allow for a fair comparison across brain areas. However, changing the analyses windows did not change the overall results of the previous studies.

RESULTS

Overview

We first describe our new data concerning the visual reference frame in FEF before quantitatively comparing FEF to our previously reported results in LIP/MIP and the SC ((Mullette-Gillman et al., 2005, 2009; Lee and Groh, 2012, 2014)). We recorded single cell response profiles while the monkeys performed visually guided saccades, interleaved with auditory guided saccades which were not analyzed here (see Methods). The task and the location of the stimuli are described in figure 2A,B. We tested whether the receptive fields of FEF neurons (N=324) shifted with the eyes or stayed fixed relative to the head by defining a reference frame index *R* akin to the average correlation coefficient between the three curves (Methods). The reference frame index is sensitive to the alignment of the three tuning curves in eye-centered or head-centered coordinates, and is relatively robust to changes in overall response magnitude. Values range from -1 to 1, where 1 indicates perfect alignment, 0 indicates poor alignment and -1 indicates perfect negative correlation. Comparison of the eye- vs head-centered reference frame indices can reveal whether response patterns are overall better described in eye-centered coordinates (Figure 2C,D), head-centered coordinates (Figure 2E,F), or if the response patterns are not well described in either coordinate system. The latter scenario occurs when the retinal positions of receptive fields differ across eye positions but not in the way consistent with head-centered coordinates, being either apparently random (Figure 2IJ) or shifting by only part of the difference in fixation position (Figure 2GH). We will refer to these latter possibilities under the heading of “hybrid” coordinates, with subcategories of “hybrid-partial shift” and “hybrid-complex”.

A final note is that in these experiments, head, body, and world are immobile with respect to each other. Thus it is not possible to distinguish these reference frames from one another.

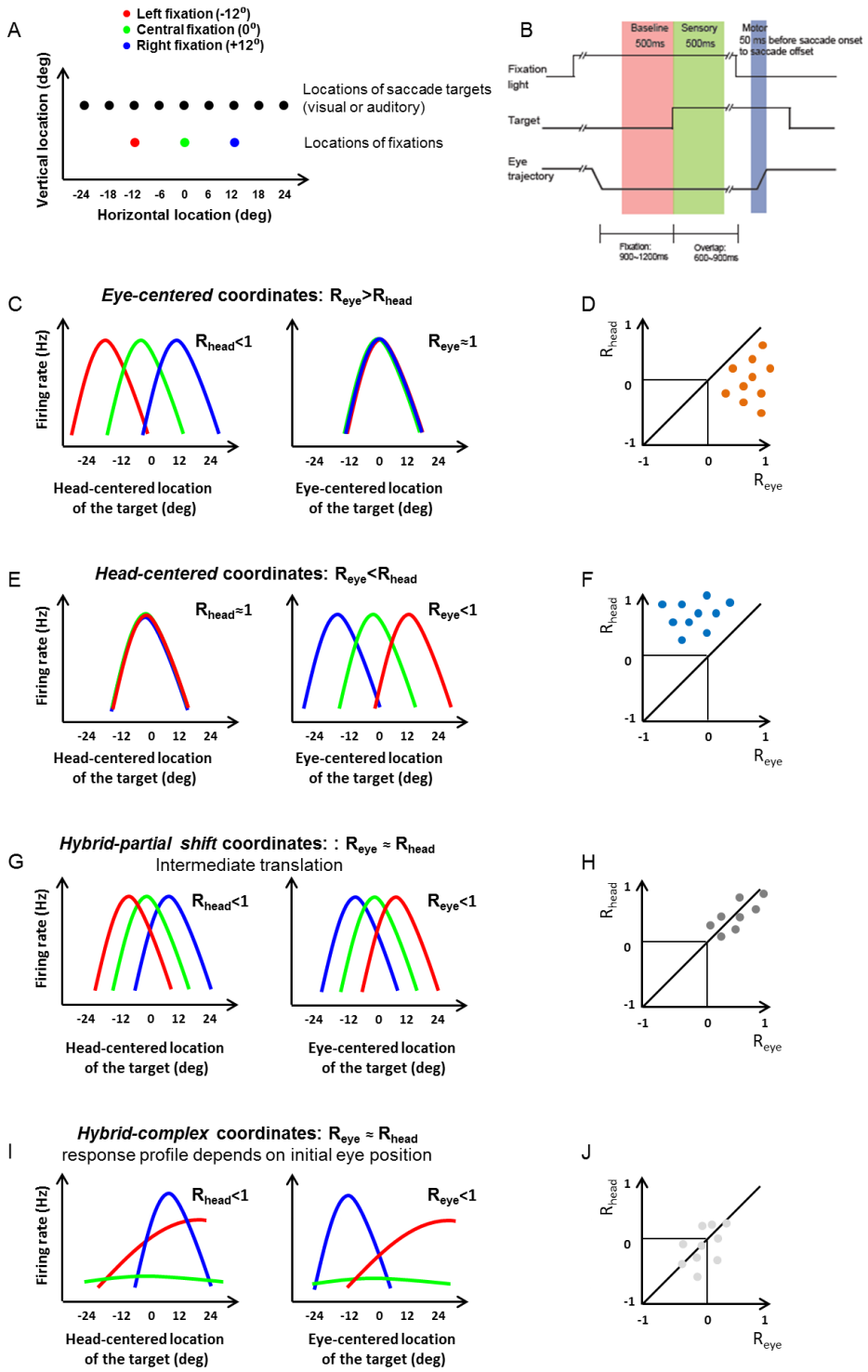

Figure 2. Schematics of task and diagrams of responses in different coordinate systems. ***(A) Locations of stimuli and initial fixations****. Varying the initial fixation permits the separation of eye- and head-centered reference frames by measuring the relative*

alignment of the responses in head- and eye-centered coordinates. ***(B) Task****: Each trial starts with the appearance of a fixation light, the monkey is required to fixate it until it later disappears. Before the offset of the fixation light, and while the monkey keeps looking at it, a target appears. When the fixation light disappears, the monkey report the location of the target by making a saccade to it.* ***(C) Pure eye-centered response pattern****. The three tuning curves obtained for the three initial fixation locations completely align in eye centered coordinates (perfect alignment, $R_{eye}\approx 1$, middle panel), while in head-centered (left panel), they are shifted by the distance between the initial eye positions (i.e. steps of 12º in the present task), resulting in $R_{head}<1$..* ***(D)*** *Position of eye-centered responses in the graph of R_{eye} vs. R_{head} (orange dots).* ***(E) Pure head centered responses****. The pattern is the opposite of (B): the three tuning curves are aligned in head-centered coordinates and separated by 12º in eye-centered coordinates.* ***(F)*** *Position of head-centered responses in the graph of R_{eye} vs. R_{head} (blue dots)* ***(G) Hybrid-partial shift response pattern****. The three tuning curves are not perfectly aligned in either head- or eye-centered coordinates: as the initial eye direction shifts left or right (in red and blue), the tuning curves only partially move apart, by less 12º.* ***(H)*** *The reference frame indices in head- and eye-centered coordinates are not significantly different from each other (dark gray dots), but at least one reference frame index value exceeds zero.* ***(I) Hybrid-complex coordinates****. The initial eye location affects both the shape and the alignment of the tuning curves.* ***(J)*** *The reference frame indices are low in either/both coordinate systems (light gray dots).*

Examples Neurons in the FEF

Figure 3 shows the responses from seven individual cells during the sensory (Figure 3 A-C-E-G) and motor periods (Figure 3 B-D-F-H). During both the sensory period and the motor burst, the most common pattern was eye-centered (Figure 3AB) with an unambiguous difference between high reference frame indexes in eye-centered coordinates (close to 1), and very low reference frame indexes in head-centered coordinates (the examples shown in Figure 3A-Bhad R_{eye} = 0.97 vs. R_{head} = -0.17 and R_{eye} = 0.93 vs. R_{head} = -0.18 respectively).

Some neurons were classified as head-centered on the basis of the quantitative comparison of the reference frame indexes (R_{head}>R_{eye}), but as can be seen in Figure 3C-D, these response patterns were not as strongly head-centered as the eye-centered neurons were eye-centered. The values of R_{head} for these two examples were 0.68 and 0.58, whereas the values of R_{eye} were 0.31 and 0.28.

There were also neurons that exhibited hybrid response patterns. The example in Figure 3E shows hybrid-complex tuning curves: two of the curves are well aligned in head-centered coordinates (the red and green ones, representing the receptive fields computed from the left (red) and central (green) fixation location), and two are well aligned in eye-centered coordinates (the green and blue ones, representing the receptive fields computed from the center and right fixation location). The example in Figure 3F shows hybrid-partial shift tuning curves: the curves clearly shift laterally when the eyes move to different initial fixation location, but not by the correct amount. In both of these examples, R_{eye} and R_{head} are about equal to each other (Figure 3E: R_{eye}=R_{head}=0.34; Figure 3F: R_{head}=0.81, R_{eye}=0.80).

A final response pattern involved responses that were only weakly modulated by target location in any reference frame. Figure 3G-H illustrates examples in which responses exceeded baseline for all locations tested, but there was little evidence of a circumscribed receptive field among the locations tested. Target-evoked responses even occurred for locations well into the ipsilateral field of space. Note that these responses were not necessarily identical for all locations, and could vary with eye position (e.g. the curves in Figure 3G,H show an overall gain sensitivity to eye position). However, the lack of spatial sensitivity in these neurons makes it impossible to evaluate their reference frame. Accordingly, we tested for spatial sensitivity using an ANOVA involving the 5 targets at -12º, -6 º, 0 º, 6 º, 12º (see Methods and Results-Overview). Neurons that failed to show spatial sensitivity in either head- or eye-centered coordinates made up about half of the sample in FEF (as well as LIP/MIP), and these neurons were excluded from the population analyses described in the next section.

Table 1. Spatially selective populations in LIP/MIP, FEF, SC.

	Number of recorded cells	Spatially selective cells in the sensory period	Spatially selective cells in the motor period	Spatially selective cells in the sensory and motor period
LIP/MIP	275	125 (45%)	127 (46%)	69 (25%)
FEF	324	174 (54%)	160 (49%)	117 (36%)
SC	179	162 (91%)	161 (90%)	153 (85%)

Table 1 legend: For each area (LIP/MIP, FEF, SC) and each time window (sensory and motor periods, see Methods) the number of spatially selectivity cells is indicated. A cell was considered spatially selective if its response was modulated by target location in either head- or eye-centered coordinates, according to two two-way ANOVAs with target location and fixation position as the two factors (one two-way ANOVA for target locations defined with respect to the eye and one two-way ANOVA for target location defined with respect to the head; target location main effects $p<0.05$; interaction terms $p<0.05$).

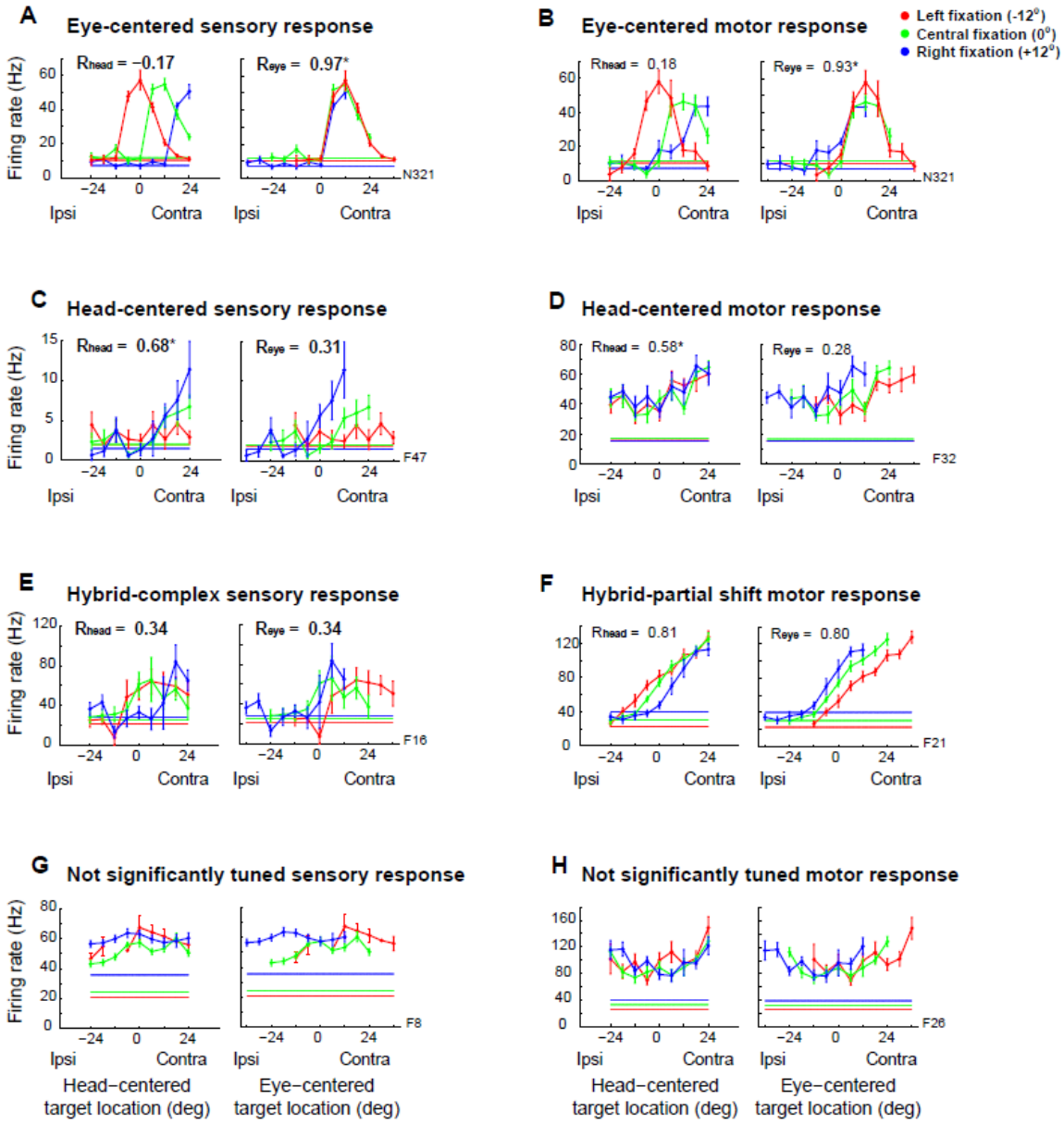

Figure 3: Examples of responses in the FEF*. Each panel shows the tuning curves for various example cells during the sensory or motor period (Methods). The tuning curves are plotted both in head-centered coordinates (left) and eye-centered coordinates (right) and the reference frame indexes R_{head} and R_{eye} are indicated.* ***(A)*** *Eye-centered responses (R_{eye} statistically higher than the R_{head}) during the sensory period.* ***(B)*** *Eye-centered responses during the motor burst (same cell as in panel A).* ***(C)*** *Head-*

centered responses (R_{eye} statistically smaller than the R_{head}) during the sensory period. ***(D)*** *Head-centered responses during the motor burst.* ***(E)*** *Hybrid-complex responses (R_{eye} not statistically different from R_{head} and both not statistically different from zero) during the sensory period.* ***(F)*** *Hybrid-partial shift responses (R_{eye} not statistically different from R_{head}, but at least one of them statistically higher than zero) during the motor burst.* ***(G)*** *Untuned responses during the sensory period.* ***(H)*** *Untuned responses during the motor burst. The reference frame index R was not calculated for the responses not significantly modulated by the target location as showed in panels G and H.*

Population Results in the Frontal Eye Fields

Eye-centered responses in the FEF are prevalent in both sensory and motor periods, corresponding to about 60% of the population. Figure 4A and B show this quantitatively. Like Figure 2D-F-H-J, these graphs plot R_{eye} vs. R_{head}. The cross hairs indicate the 95% confidence intervals on those values (see Methods). Data points are color coded orange if the 95% confidence intervals suggest $R_{eye}>R_{head}$ (the 95% range of R_{head} is lower than the 95% range of R_{eye}), blue if $R_{head}>R_{eye}$, and gray if R_{eye} and R_{head} are approximately equal; the two shades of gray correspond to $R_{eye}\sim=R_{head}$ and either $R_{eye}>0$ or $R_{head}>0$ (dark grey) and $R_{eye}\sim=R_{head}\sim=0$ (light grey). The pie charts on the right indicate the percentages of cells classified as eye-centered (orange), head-centered (blue), or the various subtypes of hybrid coding.

The pattern of reference frames was similar in the sensory vs. motor periods, and there was little evidence of a systematic change in the representation when time scale was investigated more closely. Figure 4C-D shows the average R_{eye} and R_{head} values in 100

ms time bins sliding in 50 ms increments from target onset (figure 4C) and from saccade onset (figure 4D). R_{eye} averages about 0.4 whereas R_{head} averages about 0.1, with little change except for slight upticks at target onset and saccade onset. Although R_{eye} is consistently significantly greater than R_{head} (filled symbols, t-test on each time bin, $p<0.05$), its value is not very high in absolute terms. This is because only about 60% of FEF neurons are classifiable as having eye-centered responses and among these, many R_{eye} values were not very high. On the whole, the reference frame in the FEF is more eye-centered than it is head-centered, but modestly so.

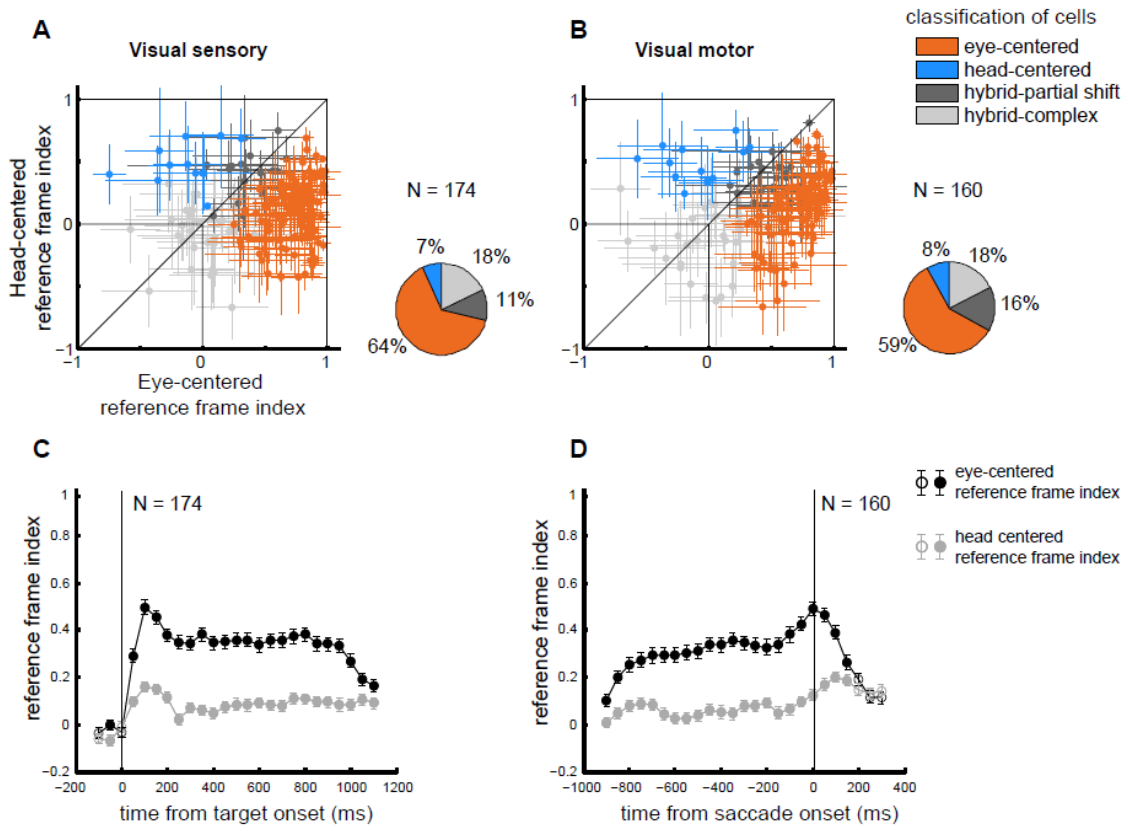

Figure 4: Reference frames in the FEF population. (A,B) *The reference frame indexes in head-centered and eye-centered coordinates are plotted for spatially selective*

cells in each time window: ***(A)*** *visual sensory,* ***(B)*** *visual motor. Responses are classified as eye centered if the 95% confidence interval of eye-centered coefficient was positive, larger than, and non-overlapping with the 95% confidence interval of head-centered coefficient (bootstrap analysis, see Methods). These are indicated in orange. Responses were classified as head centered with the opposite pattern (blue). Finally, hybrid-partial shift reference frames, in dark grey, have non-zero overlapping 95% confidence intervals, while hybrid-complex responses, in light grey, have reference frame indexes not statistically different from zero. The pie charts summarize the proportion responses classified as eye-centered, head-centered, and the subtypes of hybrid for each time window.* ***(C-D)*** *Time course of the eye-centered (black) and head-centered (grey) reference frame indexes for the FEF population of spatially selective responses (Methods). The indexes were calculated in bins of 100ms, sliding with a step of 50ms and average across the population. Trials were aligned at target onset (****C****) and at saccade onset (****D****). Filled circles indicate that the difference between the two average indexes is statistically significant (t-test for each bin, $p<0.05$).*

Comparison with LIP/MIP and the SC

We next asked how the weakly eye-centered representation in the FEF compares with the representations in parietal cortex and the SC. Figure 5 presents a comparison of the signals observed in each brain region under identical experimental conditions (see Methods and (Mullette-Gillman et al., 2005, 2009; Lee and Groh, 2012, 2014) for the description of the two additional datasets recorded in different monkey with the same technique and during the same task). As in the analysis of the FEF, we focus on two

measures. First, we compare the number of cells classified as eye-centered, head-centered, or hybrid across areas (figure 5A, B). Second, we compared the time course of the population-averaged eye-centered reference frame in the LIP/MIP, FEF, SC (figure 5C, D).

The results indicate a continuum of reference frame across these brain areas, with LIP/MIP predominantly hybrid, the SC predominantly eye-centered, and FEF intermediate between the two. In both sensory and motor periods, the proportion of eye centered responses remains a minority in the LIP/MIP (increasing from ~20% to ~40% in time) and a majority in the SC (~80% consistently in the two time periods). The FEF is between the two trends, with ~60% eye-centered responses in both time periods. Conversely, hybrid responses dominate in LIP/MIP (from ~60% to ~40%), fall to less than 40% in the FEF and to less than 20% in the SC. Head centered responses are a minority in all three areas, though a trend can be seen in both periods with the LIP/MIP having around one fifth of responses head-centered, to the FEF having fewer than 10%, to the SC having almost no head-centered signals (Figure 5A-B).

A similar picture emerges when considering the average values of the reference frame indices across time. In Figure 5CD, the average value of R_{eye} for each brain area is plotted across time (colors indicate the areas, filled dots indicate bins where the eye-centered reference frame was statistically larger than the corresponding head-centered reference frame index). Since the proportion of spatially selective cells varied in the three areas, we repeated the analysis twice: for the subpopulation of spatially selective cells (figure 5C) and for all recorded cells (figure 5D). In either population, the visual responses are significantly more eye-centered than head-centered in all areas, but with a

clear grading from SC being the most eye-centered (average index around 0.6/0.8 during the trial) to FEF (average index around 0.2/0.4 during the trial, occasionally reaching 0.5) to LIP/MIP (average index consistently lower than 0.2).

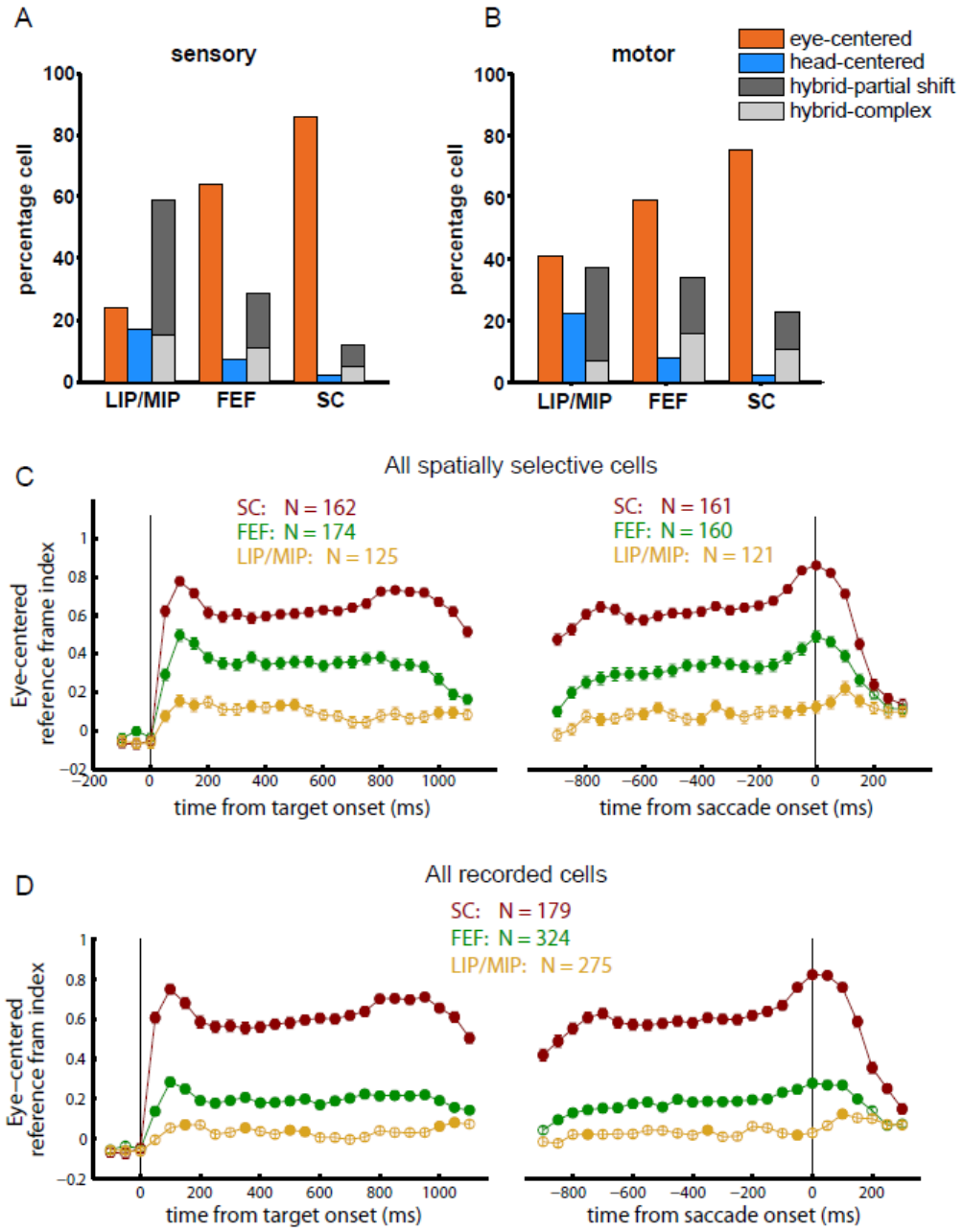

Figure 5: Reference frame indexes during sensory and motor periods in LIP/MIP, FEF and SC. **(A-B)** *The percentage of cells classified as eye-centered (orange), head-centered (blue) and hybrid (gray shades: dark gray = hybrid-partial shift, light grey = hybrid-complex) are shown for all spatially selective cells in LIP/MIP, FEF and SC in the sensory period* ***(A)*** *and in the motor period* ***(B)****. For the sensory response, the time window is the 500 ms immediately after target onset. For the motor response, the time window changes with the saccade duration, ranging from -20ms (SC), -50ms (FEF) or -100ms (LIP/MIP) from saccade onset to saccade offset (see Methods). For the FEF, the data are the same as in figure 5A, B.* ***(C-D)*** *time course of the eye-centered reference frame indexes for the populations of spatially selective cells in LIP/MIP, FEF, SC* ***(C)*** *and for all recorded cells* ***(D)****. Colors: LIP/MIP (tan), FEF (green) and SC (dark red). Filled circles indicate that the eye-centered correlation coefficient was significantly larger than the head-centered one (two-tailed t-test for each bin, $p<0.05$). The FEF data are the same as in figure 4C.*

DISCUSSION

Our quantitative assessment of the reference frame for visual space representation in the FEF shows that visual signals were mostly but not completely eye-centered. Around 60% of individual neurons were classified as eye-centered, whereas one third of neurons had hybrid reference frames and a minority had head-centered reference frames. The eye-centered reference frame index averaged about 0.2-0.4, and the pattern was largely stable across time. The pattern of reference frame in the FEF was less eye-

centered than the superior colliculus but more eye-centered than LIP/MIP, which is predominantly hybrid in its coding format.

It is a challenge to compare our results to previous studies because misinformation about the reference frames employed in the FEF and LIP/MIP pervades the literature. It is typical to find statements in abstracts or introduction sections asserting that the reference frames employed in these areas are known to be retinotopic or eye-centered, but the citations offered do not necessarily support those claims. We argue that only studies that (a) systematically varied initial eye position, (b) sampled receptive fields along the same dimension as the variation in eye position, and (c) quantified the results at the population level are relevant to this question. For a more detailed discussion of how the assessment of reference frame can go wrong when these conditions are not met, see Figures 10-11 of Mullette-Gillman et al ((Mullette-Gillman et al., 2009)).

With these caveats in mind, our FEF results are actually on the whole consistent with the most relevant previous studies. We are unaware of any single unit studies that have mapped and quantified the receptive fields at multiple eye positions in the fashion we have done here (but see (Bruce and Goldberg, 1985b; Goldberg and Bruce, 1990)). Several studies, however, have demonstrated proof of principle that many FEF receptive fields do not maintain a fixed position on the retina across eye movements (Zirnsak and Moore, 2014). The more extensive literature employing electrical stimulation shows that the direction and amplitude of the stimulation-evoked saccade can vary with initial eye position (Robinson and Fuchs, 1969; Bruce et al., 1985; Dassonville et al., 1992; Russo and Bruce, 1993; Monteon et al., 2013). Monteon et al (2013) report that about 71% of sites yield saccades of a stable vector across different eye positions (i.e. eye-centered)

and about 29 % yield saccades that vary considerably, more consistent with a head- or body-centered coordinate system.

The range of reference frames observed in the FEF provides a context for interpreting those observed in both the SC and LIP/MIP. The SC has been characterized as an eye-centered structure ((Lee and Groh, 2012) (Schiller and Stryker, 1972; Klier et al., 2001))), while the LIP/MIP is not. Mullette-Gillman ((Mullette-Gillman et al., 2005, 2009) were the first to describe the reference frame of LIP/MIP as predominantly hybrid. Andersen et al. (e.g. Andersen and Mountcastle, 1983; Andersen et al., 1990) (Andersen et al., 1985) had already reported an interaction between visual inputs and eye position in the LIP/MIP, which they interpreted as reflecting eye centered gain fields. However, their sampling of receptive fields sometimes focused on the dimension orthogonal to the change in fixation position, which made their results difficult to interpret. Adopting a more appropriate sampling technique across brain areas, we have demonstrated not only strong eye-centered signals (the vast majority in the SC) but also signals along a continuum between eye-centered and head-centered coordinates (in different proportions, in all three areas). Thus, the hybrid reference frames that we have characterized are unlikely to be due to methodological shortfalls. Furthermore, the identification, under the same conditions, of a gradual shift in the strength of eye-centered representations from LIP/MIP to FEF to SC supports the view that there is a genuine transition in coding between these different brain areas. How this observation may interact with potential differences in the breadth of receptive fields across these structures remains to be determined.

Why the brain uses reference frames that are impure, and why this should vary across brain areas is unclear. The receptive field as a labelled line for stimulus location is a concept that dates back to the discovery of receptive fields. However, if receptive fields can vary in their position, and do not show stability with respect to either eye- or head-centered coordinates, such neurons on their own cannot provide a labelled line for stimulus location. Rather, the activity of a population of such neurons would be required to disambiguate the spatial signals. While this is very possible (e.g. Pouget and Sejnowski, 1997), why it is desirable from a coding perspective has not been shown. Models of coordinate transformations show that the brain should be capable of transforming reference frames in a single step without the use of intermediate stages ((Groh and Sparks, 1992)).

An understanding of how coordinate transformations unfold in a sensorimotor context has implications more broadly, as this is any example of a many-to-many mapping that occurs in numerous other contexts. For example, numerous types of perceptual constancy involve physical stimuli that vary but produce similar percepts (space constancy, size constancy, color constancy, the same musical melody in different keys, etc.). Such constancy can require a mapping from nearly any possible range within a physical stimulus dimension to a common perceptual dimension. Similarly, categorization requires mapping different types of physical stimuli that can be grouped into a category, and associative learning involves relating two different physical stimuli in a potentially arbitrary fashion. The underlying computation supporting these abilities may be very similar to that involved with spatial coordinate transformations.

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