Supporting Information for
Sensory tuning in neuronal movement commands

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This PDF file includes:

- SI Extended Methods
- Figures S1 to S12
- SI References
SI Extended Methods

Here, we provide exhaustive details on our experimental and data analysis methods.

Laboratory setup and animal preparation

The animal laboratory setup was the same as that described in our recent work (1). Briefly, each animal was placed in a darkened room ~72 cm from a calibrated and linearized cathode-ray-tube (CRT) display (spanning approximately 30 deg horizontally and 23 deg vertically). We controlled the stimulus presentations and data acquisition procedures using a custom-built modification of PLDAPS (2), interfacing with the Psychophysics Toolbox (3-5) and an OmniPlex data acquisition system (Plexon, inc.).

We prepared the animals for behavioral training and neurophysiological experiments described earlier (1, 6, 7). Briefly, in each animal, we implanted a head holder, to stabilize head position, and a scleral search coil in one eye (8), to allow tracking eye movements with high quality using the electromagnetic induction technique (9). We also implanted a recording chamber centered on the midline and tilted 38 deg posterior of vertical. In monkey A, we positioned the chamber to allow access to the SC in the upper half of the chamber and dorsal portions of V1 in the lower half. SC and V1 recordings were performed in the left hemisphere of monkey A. Monkey M’s SC recordings were performed in both hemispheres.

We recorded neuronal activity using 16- or 24-channel linear electrode arrays with 50 µm inter-electrode spacing (V-Probes from Plexon, inc.).

Behavioral tasks

Our primary behavioral task was the “Saccades-to-X” paradigm. This was a modified version of the classic delayed, visually-guided saccade task, with the main difference being that we used an image as the eccentric saccade target rather than just a small spot of light.

Each trial started with the presentation of a white fixation spot (10.8 by 10.8 min arc dimensions) presented at display center. The spot had a luminance of 79.9 cd/m², and it was presented over a gray background of luminance 26.11 cd/m². After the monkey fixated the spot stably for 300-700 ms, an image was presented at an eccentric location. The monkey was instructed to maintain fixation on the spot even after the onset of the eccentric image. After 500-1000 ms of successful gaze fixation, the central fixation spot was removed, instructing the monkey to generate a saccade towards the center of the eccentric image. To minimize saccade vector variability across trials, which was critical for ruling out metric and kinematic changes in the saccades as the main sources of our results, we always provided a clear visual marker at the center of the eccentric image, which served as an anchor for directing the saccades towards. This marker was superimposed on the eccentric image, and it consisted of a white spot, just like the fixation spot, surrounded by a gray disc (0.54 deg diameter) of the same luminance as the background. The surrounding gray disc ensured that the marker spot was visible irrespective of the background image, allowing us to experimentally control, as much as possible, trial-to-trial variability of the saccades made to the extended images. This was important because saccades can span a range of locations on an extended foveated image (10), complicating the interpretation of whether motor burst changes were due to the image or due to different saccade vectors activating different parts of movement-related response fields (mRF’s). In subsequent post-hoc analyses (see below), we further controlled trial-to-trial variability of the saccades, again to rule out a trivial motor variance explanation of our results. The monkey was rewarded for successfully generating a saccade towards the image center within 500 ms from fixation spot offset, as well as for maintaining gaze
on the eccentric image center for an additional 500 ms. The reward amount was always the same regardless of what image was presented on a given trial.

In different blocks of trials, we used different series of images as the eccentric saccade targets. For example, in Saccades-to-Spatial-Frequency, the saccade target image consisted of a disc of 3 deg radius, the inside of which was a vertical, stationary sine wave grating of 100% contrast and a specific spatial frequency. The spatial frequency was randomly picked for each trial from among the following values: 0.5, 1, 2, 4, and 11 cycles/deg (cpd).

In the Saccades-to-Contrast variant of the task, the grating image was still vertical like in Saccades-to-Spatial-Frequency, but it now had a fixed spatial frequency (1 cpd). Across trials, we varied the contrast of the grating from among the following values: 100%, 50%, 25%, 10%, and 0%. Note that the 0% contrast grating had no luminance variation in it at all, but the marker spot (described above) was still visible at its center. Therefore, the 0% contrast condition looked identical to a classic delayed, visually-guided saccade task with a small spot as the saccade target. In fact, we used this condition as our “spot” condition in Results. Also note that because high spatial frequencies are also associated with reduced visibility (due to the contrast sensitivity function) as well as weakened SC visual responses (11), the 11 cpd condition from the Saccades-to-Spatial-Frequency paradigm above was also visually quite similar to a classic spot paradigm; it was thus more like a broad-band stimulus rather than a narrow-band one. This explains some of the neuron preference histograms of early stimulus-evoked visual bursts that are shown in Results (e.g. Fig. 3D, E, visual epoch).

We also ran a Saccades-to-Orientation version of the same task. In this case, both the spatial frequency (1 cpd) and contrast (100%) were fixed across trials. However, the grating could have different orientations. We tested 4 orientations across trials, with the following convention of defining orientation: horizontal was defined as 0 deg, and 45, 90, and 135 deg, respectively, were counterclockwise rotations from horizontal.

For Saccades-to-Objects, we were interested in whether our recent observation of visual object detection in the SC (1) extended to the saccade motor burst epoch. We had images of objects as the eccentric images. Each image was now in a square shape rather than a circular aperture as in the task variants above, and we actually analyzed the motor burst data from the same experiments conducted for our recent work (1). In that work, we only analyzed the visual burst data at image onset; here, we analyzed the saccade motor bursts. In each session, we had a total of seven natural images, one from each of seven object categories: human face, human hand, monkey neutral face, monkey aggressive face, snake, fruit, and artificial object. We also had another seven images with a grid of horizontal and vertical lines in front of them (as if the object was behind a wire mesh); another seven images with the phase information scrambled but the spatial frequency and luminance content unaltered; and another seven images with grid scrambling, or a random reshuffling of the grid locations from the image with the objects behind a grid of horizontal and vertical lines (1). The procedures for obtaining all 28 images were described recently (1), and we used existing toolboxes (12) to equalize the images in terms of luminance and spatial frequency content (1). Thus, in total, the monkey made saccades to one of 28 different images in this version of the paradigm. Across sessions, we generated new images that were not used in the previous sessions.

In all of the above task variants, there was a forced delay between target onset and saccade onset. However, in natural behavior, image onsets might “reflexively capture” saccades immediately, meaning that the visual and motor SC bursts occur much closer to each other in time. Therefore, to test the generalizability of our results to immediate, visually-guided saccade situations, we designed a second Saccades-to-X paradigm, but now without a forced delay. The monkey fixated a central fixation spot. After 600-1500 ms, the fixation spot was removed, and an eccentric gray target appeared simultaneously. We used a new feature dimension in this variant of the task, in order to demonstrate the robustness of the phenomenon of sensory tuning in SC neuronal movement bursts. The feature that we used this time was luminance contrast polarity:
targets darker than the background were defined as negative polarity stimuli, and targets brighter
than the background were defined as positive polarity stimuli (13, 14). The targets consisted of
discs of 0.51 deg radius, and they had one of three absolute Weber contrasts (10%, 50%, and
100%). Thus, in this Saccades-to-Luminance-Polarity task, we had six conditions: two luminance
polarieties (dark versus bright), and three Weber contrasts per polarity. Also note that in this task,
we did not provide a central marker spot on the discs as in the above tasks, and this was
because the discs were smaller than the other images already; we chose such smaller discs to
demonstrate that the results from the above experiments were not specific to only larger images.
Visual bursts from this task were analyzed in our recent study (14), but saccade-related motor
bursts were not inspected.

We collected 50 repetitions per condition per session from the Saccades-to-Spatial-Frequency,
Saccades-to-Contrast, Saccades-to-Orientation, and Saccades-to-Luminance-Polarity tasks, and
we collected 30 repetitions per condition per session from the Saccades-to-Objects task (because
of the increased number of conditions in this task). We typically collected Saccades-to-Spatial-
Frequency, Saccades-to-Contrast, and Saccades-to-Orientation within the same session.
However, Saccades-to-Objects required dedicated sessions due to the larger numbers of trials
that were needed. The Saccades-to-Luminance-Polarity tasks were run, largely, in separate
sessions as parts of other experiments, like those described in our recent work (14). Finally, for
the V1 recordings, we ran the Saccades-to-Spatial-Frequency, Saccades-to-Contrast, and
Saccades-to-Orientation tasks, and in simultaneous recording sessions of both the SC and V1;
the gratings were placed such that overlapping visual response fields (RF’s) of the recorded
neurons were visually-stimulated.

In all cases, we placed the saccade target at our estimated best RF and/or mRF hotspot location
of the recorded neurons, and we maintained its position throughout a block. This meant that we
ran RF mapping tasks before the main paradigms. These tasks were often the classic delayed,
visually-guided saccade task or a fixation variant of it, in which no saccade at the end of the trial
was required (1). We sometimes also ran memory-guided saccades (with target location defined
by a small spot of light). The primary reason for running these saccades was to check for a
dissociation of SC motor burst properties from movement kinematics (15), which was a relevant
point to make for the current study. However, we also used results from this dataset here as well
for some of our analyses (e.g. Fig. 6 and Fig. S11). Our choice of grating size in the main
experiments was to approximately fill classic SC visual RF’s at the eccentricities that we tested.
For simultaneous SC and V1 recordings, this meant that the grating was larger than V1 RF’s,
since we observed that V1 visual RF’s were smaller than in the SC. However, the gratings still
very robustly activated V1 neurons, as seen in Fig. 7 in Results.

Eye movement data analysis

We detected saccades in all trials using our established methods (16, 17).

To ensure that we were comparing neuronal activity with similar saccadic execution across all
different image types within a given paradigm (e.g. Saccades-to-Contrast), we first ensured that
saccade vectors were matched across the image types of the block before proceeding with any
neuronal comparisons. This is because SC movement-related RF’s are organized topographically
(18, 19); therefore, if one image systematically elicited slightly different saccade vectors from
another image, then two different parts of a given neuron’s movement-related RF would be
activated by the two images, rendering any differences in motor burst strengths trivially explained
by a difference in the saccade vectors. As a result, we always first ensured that we were
comparing motor bursts for vector-matched saccades across all image manipulations within a
given task block. For example, for Saccades-to-Contrast, we collected all saccades for each
image contrast. We then binned all vector endpoints of the saccades into a binning grid with 0.5
deg resolution (in each of the horizontal and vertical directions). We only included trials into any
subsequent analyses if a given vector bin had saccades from all image types in the blocks (i.e. all
contrasts in the example of Saccades-to-Contrast). In our example of the Saccades-to-Contrast task, if a binning grid location had only saccade vectors from low contrast images but not high contrast images, then this would mean that the saccade vectors for low and high contrasts were slightly different from each other. These saccades were, therefore, excluded from any further analyses. Because we provided a central marker on most images to guide the saccades during the experiments, we still ended up with sufficient trial repetitions for the analyses after the vector matching procedures, as evidenced by the individual example trial rasters shown in Figs. 1, 4 and Figs. S4, S7.

To further rule out subtle systematic differences between saccades to one image type versus another as the trivial explanation of our results, after matching the vectors of the saccades per the above procedure, we proceeded to analyze the movements’ metrics and kinematics across image conditions. For metrics, we calculated the direction error and the amplitude error of each saccade. Direction error was defined as the angular difference between the vector of the executed saccade and the vector of the image center relative to fixation; amplitude error was defined as the difference in radial amplitude between the vector of the executed saccade and the radial eccentricity of the image center (the saccade target). For kinematics, we calculated saccadic peak velocity. For a given saccade amplitude (as in our task design), the peak velocity should be relatively constant because of the well-known saccadic main sequence relationship (20) (the monkeys were equally rewarded across trials, so other variables that influence saccade speed, like reward, were equalized). Thus, if the peak velocity is similar for saccades to different image types and the SC motor bursts are very different, then this represents a clear dissociation between SC neuronal activity at the time of saccades from the control of saccade execution, as we and others also observed earlier (15, 21).

To analyze catch-up saccades (Fig. S2), we collected the very first saccade to occur after the primary movement had ended. Some trials did not have any catch-up saccades until the monkey was rewarded. These trials were discarded. For the rest, we calculated the onset time of the first catch-up saccade relative to the end of the primary movement. We also calculated the saccade’s amplitude, peak velocity, and direction relative to the direction of the primary saccade. Within each task (e.g. Saccades-to-Contrast), we plotted the distributions of catch-up saccade properties in different colors for the different image features that were tested.

For Saccades-to-Objects, analyses of saccade metrics and kinematics (e.g. Fig. 4D, E and Fig. S9) convinced us that the eye movement properties were already well matched across conditions. Therefore, we analyzed all trials in this task, skipping post-hoc vector matching filters. This was useful because there was a larger number of conditions to run in this task, and because vector matching might have caused severe biases in which images were included or removed as opposed to others in a given analysis.

As we describe in more detail below, we typically grouped trials according to the SC motor burst strength. For example, if a neuron had the strongest motor burst for a high contrast image as the saccade target, we defined this image as the “most preferred image” for the “motor burst”. Similarly, if the same neuron had the weakest motor burst for a low contrast image, then this image was the “least preferred image” for the “motor burst”. With such classification, we could compare saccade metric and kinematic properties for the most and least preferred trials. Therefore, we also analyzed the saccades of such trials. We always showed full distributions of our data points, and we also included descriptive statistics (e.g. mean or median values). For kinematic comparisons between most and least preferred images, we additionally calculated a kinematic modulation index. This index was defined as the peak speed of the eye for saccades to the most preferred image minus peak speed for saccades to the least preferred image, divided by the sum of peak speeds. Thus, if the kinematic modulation index was zero, it meant that saccade peak speed was the same for trials with the most and least preferred images. For each neuron, we had a kinematic modulation index from its sessions’ saccades, which we compared to a neuronal modulation index described below. Note that we often recorded multiple neurons simultaneously. However, since different neurons could have different most and least preferred
images, the saccades used for computing kinematic modulation indices (or for other plots of saccadic behavior) were not necessarily the very same saccades for multiple simultaneously recorded neurons.

Neuronal data analysis

We sorted individual neurons offline using the Kilosort Toolbox (22), followed by manual curation using the phy software. We then proceeded to analyze spike times and firing rates in the different conditions. To obtain firing rates, we convolved spike times with Gaussian kernels of σ 10 ms.

Our primary goal was to analyze saccade-related “motor” bursts in the SC. To do so, we defined a motor burst epoch as the time interval between -50 ms and 25 ms from saccade onset, in which we measured average firing rates. For comparison, we also analyzed stimulus-evoked visual bursts occurring immediately after image onset. For those, we defined a visual burst epoch as the time interval between 50 ms and 150 ms after image onset during gaze fixation, and we measured average firing rate in this interval.

For classifying SC neurons into different functional cell types (e.g. visual, visual-delay, visual-motor, or motor), we also had additional measurement intervals. The baseline interval was defined as -100 to 0 ms relative to image onset at trial beginning, and the delay-period interval was 400-500 ms after image onset. Within each task variant that we analyzed (e.g. Saccades-to-Contrast), we classified each neuron as being predominantly visual (exhibiting only stimulus-evoked visual bursts), predominantly motor (exhibiting only saccade-related bursts), visual-motor, or visual-delay (exhibiting visual and saccade-prelude activity but no significant motor activity); see Fig. 5A for examples. To do so, we used the measured firing rates in the four measurement epochs described above (baseline, visual epoch, delay epoch, and motor burst epoch) and computed a non-parametric ANOVA (Kruskal-Wallis). We then determined neuronal class by post-hoc tests at the p<0.05 level. Very few well-isolated neurons were not classified into any of the above categories, whether due to low activity levels or other reasons causing the statistical tests to fail. In our population analyses pooling all neuron types, we also included these minority unclassified neurons because inspecting them revealed the same patterns as those of the well-categorized neurons. Also note that we classified neurons separately in each task variant because our primary goal was to ask whether sensory-tuning in SC neuronal movement commands was robust even in neurons with relatively stronger movement-related rather than visual-related activity (e.g. Fig. 5) within any given task. The question of whether SC mRF’s themselves are different for different image types is orthogonal to this investigation and requires dedicated mRF mapping sessions with multiple image types (a technically-challenging endeavor due to the numbers of trials required). Moreover, there could be task-related variability in firing rates (e.g. differences in delay-period activity) across different stimulus types. Finally, our neuron classification was highly robust across the different tasks, as evidenced by the similarity of our visual-motor indices (described below) across tasks (e.g. Fig. S10A).

For analyzing SC saccade-related “motor” bursts, in each task variant (e.g. Saccades-to-Contrast), we defined (for each neuron) the image associated with the strongest saccade-related motor burst as the "most preferred" image. We also defined the image associated with the weakest saccade-related motor burst as the "least preferred" image. This was done after the vector-matching procedures described above. Because different neurons had different preferred and non-preferred images (see Results), this classification allowed us to obtain population-level effect sizes across neurons. To do so, we normalized each neuron’s firing rate and then averaged across neurons. Normalization was done as follows. In each neuron, we found the peak firing rate occurring either after stimulus onset or around saccade triggering. We then used the larger of the two peaks and subtracted the neuron’s baseline activity from it. This constituted our normalization constant. For any firing rate that we wanted to normalize in the neuron’s data, we subtracted the neuron’s baseline activity from it and then divided by the baseline-subtracted maximal response of the neuron (i.e. by our normalization constant). After obtaining the population saccade-related...
motor burst strengths for the most and least preferred images, we plotted the differences between them as well (e.g. Fig. 2A, gray).

We also calculated neuronal modulation indices, similar to what we did with the kinematic modulation indices described above. For each neuron, we plotted the average firing rate curve of the neuron around saccade onset. We then measured the maximum of this curve in the interval from -50 to 25 ms relative to saccade onset, and we did this for either the most or least preferred feature. The neuronal modulation index was defined as the burst strength of the neuron for the most preferred feature minus the burst strength for the least preferred feature divided by the sum of the two. We then plotted the distributions of neuronal modulation indices across neurons in our different tasks. To compare neuronal modulation indices to kinematic modulation indices, we plotted the two indices against each other for each task, and we calculated correlation coefficients. This allowed us to assess whether a large change in burst firing rate in a given neuron (e.g. Fig. 2A) was associated with an equally large change in saccade kinematics or not.

Because we found that the most and least preferred images could be different within a single neuron between the early stimulus-evoked visual burst epoch and the saccade-related motor burst epoch (e.g. Fig. S7A, B), we also repeated the above procedure for the early “visual” epoch of the trials (immediately after image onset during fixation), but after first identifying the most and least preferred images of each neuron in this visual epoch. The normalization of firing rates was not re-done since the above normalization was applied to entire trials and not just the motor burst epochs.

To compare the distributions of preferred features in the early stimulus-evoked visual burst epoch and the saccade-related motor burst epoch (e.g. Fig. 3D-F), we performed $\chi^2$ tests for each image manipulation. We also did this for the reflexive version of the saccade task (Saccades-to-Luminance-Polarity; Fig. S6).

We also performed comparisons on raw firing rates, either by plotting the raw measurements directly (e.g. Fig. 2B), or by using receiver-operating-characteristic (ROC) analyses in a manner similar to other studies (23). We calculated the area under the ROC curve (AUC) as a function of time in either saccade or stimulus-evoked visual epochs. For each trial of the “most preferred” image, we measured instantaneous firing rate at a given point (e.g. near saccade onset), and we did the same for the “least preferred” image. We then calculated the AUC across the distribution of trials at that time point. We repeated this procedure as a function of time, and this gave us time courses of AUC changes relative to either saccade onset or stimulus onset. This procedure allowed us to demonstrate differences in saccade-related bursts despite matched saccade vectors, and it is similar to analyses performed for pre-saccadic elevations in visual cortical neurons of area V4 (23). In some analyses, we also performed AUC analyses as a function of identified cell type. Here, we used the classification of neurons described above and performed the analysis only on neurons within a given functional cell class. We then performed a statistical test to evaluate whether the AUC value at saccade onset depended on cell type (e.g. Fig. 5). Specifically, for each peri-saccadic AUC curve in one task (e.g. Saccades-to-Contrast), we measured the average AUC in the interval from -20 to +20 ms from saccade onset, and we did this for each classified cell type. Then, we ran a non-parametric ANOVA (Kruskal-Wallis) to assess whether cell type influenced the value of the peak peri-saccadic AUC or not. We then performed post-hoc comparisons between pairs of cell types. Our AUC calculations were similar to those we used recently (1).

For Saccades-to-Objects, we were particularly struck by the preference of saccade-related motor bursts to real object images as opposed to scrambles (e.g. Fig. 4F). Therefore, we checked whether neurons had significant peri-movement AUC elevations when comparing object images to scrambled images. Thus, we grouped all seven image categories into one. This resulted in four groups: real objects, grid-covered real objects, phase-scrambled objects, and grid-scrambled objects. We then checked for significant peri-movement AUC values when comparing real objects to either phase or grid-scrambled categories (or both). We assessed significance, similarly to how
we did it recently for SC visual responses to objects (1). Specifically, we calculated bootstrapped confidence intervals for AUC measures; neurons that had AUC values significantly different from 0.5 at the p<0.05 anywhere from times -100 to 100 ms relative to saccade onset were deemed to be significant.

For local field potential (LFP) analyses, we obtained raw wide-band signals from each electrode contact. We then applied zero-lag filtering procedures as described previously (24). Briefly, we used notch filtering to remove the line noise frequency (50 Hz) and its next two harmonics (100 and 150 Hz), and we also kept signals <300 Hz as the LFP band. To classify whether the channel from which we collected LFP’s was from the more visual (superficial) or more motor (deep) SC layers, we classified each electrode channel’s multi-unit activity (MUA) as being predominantly visual or predominantly motor using a visual-motor index (VMI) (25, 26). Specifically, for each channel, we filtered the wide-band signal using a fourth-order Butterworth band-pass filter (750 to 5000 Hz), and we then rectified the signal before passing it through a second low-pass filter (fourth-order Butterworth) with 500 Hz frequency cutoff. For each condition, we plotted stimulus- and saccade-aligned MUA responses after subtracting the baseline MUA level (defined as the average MUA in the final 200 ms before image onset); superficial channels had stronger visual than motor responses, whereas deeper channels had stronger motor than visual response (25, 26). To quantify this, we measured a motor MUA value and a visual MUA value. These were defined as the average baseline-subtracted MUA in the interval -25 to 25 ms from saccade onset (for the motor MUA measurement) or 30 to 200 ms after image onset (for the visual MUA measurement). The VMI was defined as the motor MUA measurement minus the visual MUA measurement divided by the sum of the two. VMI’s larger than zero were more motor than visual (e.g. Fig. S10A).

For state-space analyses, we performed a pseudo-population analysis (27, 28). For each task, the instantaneous firing rate of all neurons that we recorded from was a point in an N-dimensional space of the activity of the population of N neurons. As all neurons’ firing rates changed across time (e.g. after stimulus onset or peri-saccadically), the population activity representation moved in this N-dimensional space. We, thus, assumed stability across sessions of SC activity since not all neurons in our population were recorded simultaneously (27). Since population activity likely occupied a much lower dimension than the number of neurons, we performed principal components analysis (PCA) and plotted the population trajectory within the first 3 PCA dimensions. These typically accounted for the majority of the variance of population firing rates (e.g. 70-89%). We used such state-space analysis to first compare visual and motor burst population trajectories and then to check for tuning in the motor bursts. In both cases, we normalized each neuron’s firing rate before performing PCA, using the same normalization procedure described above.

To compare visual and motor burst population trajectories in PCA space, we concatenated each neuron’s activity in a visual interval (from 0 to 200 ms relative to stimulus onset) with activity in a saccade epoch (from -100 to 50 ms relative to saccade onset). Then, we projected the population activity on 3-dimensional PCA space. This allowed us to assess whether visual and motor SC activity occupied similar or different subspaces (e.g. Fig. 3G).

To check for sensory tuning in the motor bursts themselves, we focused on the peri-saccadic interval only, and we projected SC population activity in this interval, using PCA, for different images as the saccade targets. If there was sensory tuning in the SC population motor bursts, then the population peri-saccadic state-space trajectories should differ as a function of which image was the saccade target (despite the vector- and kinematically-matched saccades) (e.g. Fig. 3H). To quantitatively confirm such difference, we then picked a reference peri-saccadic trajectory from one of the image features of the experiment (e.g. spot in the Saccades-to-Contrast experiment), and we calculated the Euclidean distance of each other image feature’s population activity trajectory from this reference trajectory. We did this for times around saccade onset. Moreover, we calculated Euclidean distances from the entire high-dimensional population space, and not just from the 3-dimensional PCA sub-projection of only 3 principal components. For
checking Euclidean distances against a null distance distribution, we performed 1000 permutations in which we randomly picked a reference and a condition trajectory, and we then calculated the Euclidean distance between the two.

Analyses of V1 visual responses were similar to those of SC visual responses, except that our measurement interval was 30 to 150 ms after stimulus onset, since we observed that V1 neurons had slightly earlier visual response latencies than SC neurons.

Finally, in our analysis of memory-guided saccades from past experiments (15), we replotted the same neuronal modulation indices calculated earlier (15) but now as a histogram distribution (Fig. 6A). The modulation indices involved measuring the motor burst for vector-matched saccades towards either a spot or a blank. They were calculated as indicated in the equation in Fig. 6A (15).
Fig. S1. Independence of sensory tuning in SC neuronal movement commands from eye movement metric properties. (A) For each image manipulation of Fig. 2 (different colors), and for each neuron (individual symbols), we plotted the amplitude error of the saccades to the most and least preferred images of the neuron (based on its motor burst strengths). Despite the large differences in saccade motor bursts (Fig. 2), the amplitude errors were similar across images. This confirms our vector matching procedures (Materials and Methods). P-values indicate rank-sum test results from each image manipulation. (B) Distributions of amplitude error differences between most and least preferred image trials from A. The violin plots always straddled zero. (C, D) Similar observations with saccade direction errors. The numbers of neurons indicated apply to A, B as well.
Fig. S2. Independence of sensory tuning in SC neuronal movement commands from the properties of catch-up saccades after the primary movements. (A) For the spatial frequency image manipulation, the leftmost panel shows the distribution of catch-up saccade onset times after the end of the primary saccade. Each color shows a specific image feature to which the primary saccade was directed. The vertical lines indicate the means of the individual distributions. The second plot shows similar distributions but for catch-up saccade amplitude, and the third plot does it for catch-up saccade peak velocity. The final polar plot shows the distribution of catch-up saccade directions relative to the direction of the primary saccade (that is, a direction of zero in the plot would indicate that the catch-up saccade was in the same direction as the primary saccade). In all panels, the distributions of catch-up saccades were very similar to each other for the different image features, despite the differences in SC motor burst strengths that we observed (Figs. 1, 2). (B) Similar observations for the contrast image manipulation. (C) Similar observations for the orientation image manipulation. Note that our other tasks (Materials and Methods) also had similar properties of catch-up saccades.
Fig. S3. Independence of sensory tuning in SC neuronal movement commands from intrinsic image salience, as inferred from saccadic reaction times. (A-C) In each of the spatial frequency, contrast, and orientation image manipulations, we used a delayed-saccade paradigm to enforce a period of steady-state gaze fixation between image onset and saccade triggering. This allowed us, as much as possible, to equalize saccadic reaction times across image features within each image manipulation, as can be confirmed from the strongly overlapping saccadic reaction time histograms in each panel. Thus, even though some image features, like low spatial frequencies (11), might be more intrinsically salient than others we equalized this as much as possible by our delayed-saccade paradigm. Note also that in our luminance polarity image manipulation, we used reflexive saccades instead, and the results were unchanged despite strong differences in saccadic reaction times with different image features (see Fig. S4). Vertical lines indicate mean reaction times for each image feature.
Fig. S4. Independence of sensory tuning in SC neuronal movement commands from reflexive versus delayed saccades. (A) An example neuron’s firing rates from our luminance polarity image manipulation. In this image manipulation, we avoided delayed saccades, and the monkeys reflexively looked at the peripheral stimulus as soon as it appeared. This example neuron had almost non-existent visual responses to stimulus onset, but it had strong motor bursts. For two different image features, the motor bursts were very different, similar to the example neuron of Fig. 1. Error bars: 95% confidence intervals, and the numbers of trials are indicated by the number of spike raster rows shown. Other conventions are similar to Fig. 1, and the colors indicate the individual image features, as per the legend in C. (B) A second example neuron possessing both visual and motor bursts. Note how the visual burst had strongly different latencies from stimulus onset in the two shown conditions, which was also reflected in different saccadic reaction times (C). Nonetheless, the motor bursts were still sensory-tuned like in the delayed-saccade paradigm of Fig. 1. Also note how the neuron flipped its image feature preference between visual and motor burst epochs, showing weaker visual bursts but stronger motor bursts for the same image. This is consistent with a transformed SC representation of images at the time of saccade triggering (also see Fig. 3 and Figs. S6-S8). Error bars: 95% confidence intervals, and trial numbers can be inferred from the shown spike rasters. (C) With the reflexive saccade paradigm used in this image manipulation, saccadic reaction times strongly depended on image contrast, and there were modulatory effects of luminance polarity (14). Thus, whether saccadic reaction times were equalized (Fig. S3) or not (this figure), sensory tuning in SC neuronal movement commands was still robustly observed. Vertical lines indicate mean saccadic reaction times for each image feature.
Fig. S5. Sensory tuning in SC visual responses. (A) Analyses like those in Fig. 2A, but for the visual responses of the neurons rather than their activity at the time of saccade triggering. The differences in firing rates between most and least preferred images in the visual bursts were smaller than in the saccade bursts of Fig. 2A, consistent with the AUC discrimination performance results documented in Fig. 3A-C. (B) Neuronal modulation indices from the visual burst epoch; these were calculated similarly to the modulation indices in the motor bursts, but based on visual burst measurements and feature preferences (Materials and Methods). All conventions are similar to Fig. 2. Also note that the luminance polarity image manipulation was from the reflexive saccade paradigm. Therefore, there were secondary elevations in firing rates after the initial visual responses in A, reflecting the saccade motor bursts. Error bars: 95% confidence intervals, and neuron numbers are indicated in A.
**Fig. S6.** Similar observations to Fig. 3A-F during the reflexive saccade paradigm. (A) From the luminance polarity image manipulation, we plotted peri-saccadic AUC discrimination performance across neurons. Consistent with Fig. 3 and the example neurons of Fig. S4, there was a peak in AUC discrimination performance at the time of SC motor bursts. (B, C) Also consistent with Fig. 3, the distribution of preferred image features at saccade onset (C) was broader than that at stimulus onset (B), suggesting amplification of weak visual signals at the time of saccade generation even with reflexive, visually-guided saccades. This difference in distributions was statistically significant ($p=7.4\times10^{-6}$; $\chi^2=31.4927$; $\chi^2$ test). Figure S4B shows an example neuron with such amplification at the time of saccades. Also see Fig. S8 for high-dimensional population activity trajectories in the reflexive saccade paradigm.
Fig. S7. Potential transformation of image preferences between stimulus and saccade onsets in individual SC neurons. (A-C) Three example neurons from our three image feature manipulations with the delayed saccade paradigm, demonstrating how a changed feature preference can occur between visual and motor epochs with simple grating stimuli. Note how the weak signals in the visual epochs in A, B were transformed into stronger motor bursts at the time of saccade triggering. Also note that this is similar to the example neuron of Fig. S4B in the immediate, reflexive saccade paradigm. The neuron in C, on the other hand, did not exhibit an altered preference between its visual and motor epochs. Error bars: 95% confidence intervals, and trial numbers can be inferred from the shown spike rasters.
Fig. S8. Embedding of image feature information in SC population activity at the time of saccades. (A) Monkey A population activity trajectories in the first 3 principal components after PCA decomposition in the contrast, orientation, and luminance polarity image manipulations (spatial frequency was shown in Fig. 3H). Consistent with Fig. 3H, SC neurons occupied different manifolds in population activity space at the time of saccade triggering for different image features. Note that in luminance polarity, the saccades were reflexive. Thus, visual and motor bursts occurred in close temporal proximity to each other. Nonetheless, their transformation into quasi-orthogonal manifolds between the visual and motor epochs was still visible, consistent with Fig. 3G. (B) For each image manipulation, we picked a reference condition (spot for contrast, 135 deg for orientation, and 100% dark for luminance polarity), and we then plotted the peri-saccadic Euclidean distance of high-dimensional SC population activity from this condition at the time of saccade generation. In each case, the Euclidean distances were different for different image features, suggesting the embedding of sensory information at the time of SC motor bursts (despite vector and kinematic saccade matching). Note that for luminance polarity, the sustained elevation of Euclidean distances before saccade onset reflects the visual epochs of this reflexive saccade task, which were also sensory-tuned. (C, D) Similar results from monkey M. Also see Fig. S9D-G for consistent results of sensory tuning in SC neuronal movement commands with real-life object images.
Fig. S9. Real-life object representations in SC neuronal movement commands. (A) Plots similar to Fig. 2B, C and Fig. S1A, C showing a dissociation between motor burst effects between most/least preferred images (top left) and saccade kinematics (top right) or saccade metrics (bottom left and right) in the experiment testing real-life object images (Fig. 4). (B, C) Distributions of saccade amplitude and direction error differences between most and least preferred images like in Fig. S1B, D, consistent with the interpretation that SC motor burst differences in this experiment were not explained by systematic differences in eye movement parameters (also see Fig. 4D, E). (D, E) Two different views of the peri-saccadic PCA-space population trajectories from both monkeys in the experiments with object images. Note how object and object+grid images (having coherent visual form images within them) were more differentiated from phase-scrambled and grid-scrambled images. (F) High-dimensional space Euclidean distances as a function of time from saccade onset when phase-scrambled images were the reference trajectory. This is a similar analysis to that in Fig. 3I, but for the real-life object experiments. Euclidean distances peaked near saccade onset, and they were consistently higher than distances obtained with randomly shuffled reference and non-reference trajectories (black +/- 95% confidence intervals). Object and object+grid images were also more differentiated (higher Euclidean distances) from phase-scrambled images than grid-scrambled images at the time of saccade triggering, consistent with Fig. 4F. (G) Similar analysis to F but with grid-scrambled images now providing the reference trajectory. Once again, the object and object+grid images were the most differentiated at the time of saccades from grid-scrambled images. All other conventions are similar to F. Therefore, whether referencing to phase- or grid-scrambled images, SC motor bursts for real-life objects were most differentiated from those for scrambled images.
Fig. S10. Embedding of sensory information at saccade onset within the deeper SC layers. (A) We calculated a visual-motor index (VMI) (Materials and Methods and refs. 25, 26) across electrode depths from the example session shown in Fig. 1. The VMI, which is inferred from multi-unit activity (MUA) near a given electrode contact, is positive for more motor layers and negative for more visual layers, and the example neuron of Fig. 1 was recorded from channel 14 (that is, from a strongly motor layer). The VMI was calculated for each image manipulation separately (3 colors), and it was robust across them. (B) Example MUA activity profiles near stimulus or saccade onset from the same example session. Responses are shown from channel 2 and channel 14, demonstrating how channel 2 was predominantly visual (no motor bursts) and channel 14 was predominantly motor (no visual bursts). (C) Local field potential (LFP) profiles around saccade onset for two example features (e.g. 0.5 and 11 cpd) from each image manipulation tested in this session (spatial frequency, contrast, and orientation). The LFP responses from channel 2 are shown. There were differences in peri-saccadic LFP responses for different image features of the saccade targets, despite matched saccade kinematics and metrics. This is consistent with the presence of sensory information in the local SC network at the time of saccade motor burst generation. Note how the effect was weakest for the orientation image manipulation. (D) Similar analyses from the deeper motor layer of channel 14 (where the example neuron of Fig. 1 was recorded). There was still a clear sensory signal in the peri-saccadic LFP responses despite the depth of the recording, consistent with the results of Figs. 1-3, 5. Again, the effect was weakest in the orientation image manipulation. (E-G) VMI, MUA, and LFP responses from another session from the real-life object experiment. Peri-saccadic LFP responses from an example motor layer (same as that of the example neuron of Fig. 4B) also differentiated between coherent and scrambled object images (G). Error bars in all cases: SEM.
**Fig. S11.** Comparison of SC motor bursts for saccades made towards either a white spot or a blank. Four additional example neurons from different parts of the distribution of neuronal modulation indices of Fig. 6A. The leftmost two neurons had much weaker peak saccade-related discharge for the blank condition than for the visible target condition. This was the case despite the fact that the neurons emitted strong motor bursts of up to almost 300 or 600 spikes/s peak discharge when the saccade target was visible. The third neuron was less strongly affected by the absence of a visual target for the saccade, and the fourth neuron was even less affected (this example represented a minority in the population, as can be seen from the distribution of Fig. 6A). Error bars denote SEM, and the saccade vectors were always matched between the visible target and blank conditions (15).
Fig. S12. Example SC neurons with visually-dependent saccade-related discharge from the database of a previous study (24). (A) Each row represents an example neuron in which we plotted the saccade-related motor RF map, from a task in which the saccade target was a white spot. The horizontal and vertical axes indicate horizontal and vertical saccade amplitudes, respectively. The z-axis denotes the average pre-saccadic firing rate (in the final 50 ms before saccade onset) emitted by the neuron. Note that peak motor burst strength typically occurs after saccade onset, suggesting that all six shown neurons had strong motor burst peaks of >100.
spikes/s. (B) For each neuron, we plotted the average peri-movement firing rate curve from all RF sample locations displayed in A. There was a clear saccade-related motor burst. Note that the shown plots under-represent the peak motor burst strength of each neuron because they included all sampled saccade vectors from A (including those outside of the RF and with minimal saccade-related discharge). Nonetheless, clear saccade-related motor bursts could still be seen. (C) We then plotted each neuron’s discharge for a single saccade vector to a location near the RF hotspot location from A. This time, the saccade was made towards a blank (memory-guided saccade). Each panel plots the eye position time course (top) and the associated average firing rate (bottom), both aligned to saccade onset. In all six cases, the neurons did not emit motor bursts with saccades towards a blank, despite being clearly saccade-related in A, B. Error bars: SEM.
References

1. A. R. Bogadhi, Z. M. Hafed, Express detection and discrimination of visual objects by primate superior colliculus neurons. *BioRxiv* 10.1101/2022.02.08.479583 (2022).
2. K. M. Eastman, A. C. Huk, PLDAPS: A Hardware Architecture and Software Toolbox for Neurophysiology Requiring Complex Visual Stimuli and Online Behavioral Control. *Front Neuroinform* 6, 1 (2012).
3. D. H. Brainard, The Psychophysics Toolbox. *Spatial vision* 10, 433-436 (1997).
4. D. G. Pelli, The VideoToolbox software for visual psychophysics: transforming numbers into movies. *Spatial vision* 10, 437-442 (1997).
5. M. Kleiner, D. Brainard, D. G. Pelli, What's new in Psychtoolbox-3? (Abstract). *Perception* 36 (2007).
6. J. Skinner, A. Buonocore, Z. M. Hafed, Transfer function of the rhesus macaque oculomotor system for small-amplitude slow motion trajectories. *J Neurophysiol* 121, 513-529 (2019).
7. K. F. Willeke et al., Memory-guided microsaccades. *Nat Commun* 10, 3710 (2019).
8. S. J. Judge, B. J. Richmond, F. C. Chu, Implantation of magnetic search coils for measurement of eye position: an improved method. *Vision Res* 20, 535-538 (1980).
9. A. F. Fuchs, D. A. Robinson, A method for measuring horizontal and vertical eye movement chronically in the monkey. *J Appl Physiol* 21, 1068-1070 (1966).
10. T. Moore, Shape representations and visual guidance of saccadic eye movements. *Science* 285, 1914-1917 (1999).
11. C. Y. Chen, L. Sonnenberg, S. Weller, T. Witschel, Z. M. Hafed, Spatial frequency sensitivity in macaque midbrain. *Nat Commun* 9, 2852 (2018).
12. V. Willenbockel et al., Controlling low-level image properties: the SHINE toolbox. *Behav Res Methods* 42, 671-684 (2010).
13. T. Malevich, A. Buonocore, Z. M. Hafed, Dependence of the stimulus-driven microsaccade rate signature in rhesus macaque monkeys on visual stimulus size and polarity. *J Neurophysiol* 125, 282-295 (2021).
14. T. Malevich, T. Zhang, M. P. Baumann, A. R. Bogadhi, Z. M. Hafed, Faster Detection of "Darks" than "Brights" by Monkey Superior Colliculus Neurons. *J Neurosci* 42, 9356-9371 (2022).
15. T. Zhang, T. Malevich, M. P. Baumann, Z. M. Hafed, Superior colliculus saccade motor bursts do not dictate movement kinematics. *Commun Biol* 5, 1222 (2022).
16. C. Y. Chen, Z. M. Hafed, Postmicrosaccadic enhancement of slow eye movements. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 33, 5375-5386 (2013).
17. M. E. Bellet, J. Bellet, H. Nienborg, Z. M. Hafed, P. Berens, Human-level saccade detection performance using deep neural networks. *J Neurophysiol* 121, 646-661 (2019).
18. D. A. Robinson, Eye movements evoked by collicular stimulation in the alert monkey. *Vision Res* 12, 1795-1808 (1972).
19. D. L. Sparks, C. Lee, W. H. Rohrer, Population coding of the direction, amplitude, and velocity of saccadic eye movements by neurons in the superior colliculus. *Cold Spring Harb Symp Quant Biol* 55, 805-811 (1990).
20. B. L. Zuber, L. Stark, G. Cook, Microsaccades and the velocity-amplitude relationship for saccadic eye movements. *Science* 150, 1459-1460 (1965).
21. J. A. Edelman, M. E. Goldberg, Dependence of saccade-related activity in the primate superior colliculus on visual target presence. *J Neurophysiol* 86, 676-691 (2001).
22. M. Pachitariu, N. A. Steinmetz, S. N. Kadir, M. Carandini, K. D. Harris, Fast and accurate spike sorting of high-channel count probes with KiloSort. *Advances in Neural Information Processing Systems (NIPS 2016)* 29 (2016).
23. T. Moore, M. H. Chang, Presaccadic discrimination of receptive field stimuli by area V4 neurons. *Vision Res* 49, 1227-1232 (2009).
24. Z. M. Hafed, C. Y. Chen, Sharper, Stronger, Faster Upper Visual Field Representation in Primate Superior Colliculus. *Curr Biol* **26**, 1647-1658 (2016).

25. C. Massot, U. K. Jagadisan, N. J. Gandhi, Sensorimotor transformation elicits systematic patterns of activity along the dorsoventral extent of the superior colliculus in the macaque monkey. *Commun Biol* **2**, 287 (2019).

26. C. Bourrelly, C. Massot, N. J. Gandhi, Rapid Input-Output Transformation between Local Field Potential and Spiking Activity during Sensation but not Action in the Superior Colliculus. *J Neurosci* **43**, 4047-4061 (2023).

27. U. K. Jagadisan, N. J. Gandhi, Population temporal structure supplements the rate code during sensorimotor transformations. *Current Biology* **32**, 1010-1025 (2022).

28. E. F. Kutter, J. Bostroem, C. E. Elger, F. Mormann, A. Nieder, Single Neurons in the Human Brain Encode Numbers. *Neuron* **100**, 753-761 e754 (2018).