Segregation of Visual Response Properties in the Mouse Superior Colliculus and Their Modulation during Locomotion

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The superior colliculus (SC) receives direct input from the retina and integrates it with information about sound, touch, and state of the animal that is relayed from other parts of the brain to initiate specific behavioral outcomes. The superficial SC layers (sSC) contain cells that respond to visual stimuli, whereas the deep SC layers (dSC) contain cells that also respond to auditory and somatosensory stimuli. Here, we used a large-scale silicon probe recording system to examine the visual response properties of SC cells of head-fixed and alert male mice. We found cells with diverse response properties including: (1) orientation/direction-selective (OS/DS) cells with a firing rate that is suppressed by drifting sinusoidal gratings (negative OS/DS cells); (2) suppressed-by-contrast cells; (3) cells with complex-like spatial summation nonlinearity; and (4) cells with Y-like spatial summation nonlinearity. We also found specific response properties that are enriched in different depths of the SC. The sSC is enriched with cells with small RFs, high evoked firing rates (FRs), and sustained temporal responses, whereas the dSC is enriched with the negative OS/DS cells and with cells with large RFs, low evoked FRs, and transient temporal responses. Locomotion modulates the activity of the SC cells both additively and multiplicatively and changes the preferred spatial frequency of some SC cells. These results provide the first description of the negative OS/DS cells and demonstrate that the SC segregates cells with different response properties and that the behavioral state of a mouse affects SC activity.

Key words: mouse; silicon probe; superior colliculus; vision

Significance Statement

The superior colliculus (SC) receives visual input from the retina in its superficial layers (sSC) and induces eye/head-orientating movements and innate defensive responses in its deeper layers (dSC). Despite their importance, very little is known about the visual response properties of dSC neurons. Using high-density electrode recordings and novel model-based analysis, we found several novel visual response properties of the SC cells, including encoding of a cell's preferred orientation or direction by suppression of the firing rate. The sSC and the dSC are enriched with cells with different visual response properties. Locomotion modulates the cells in the SC. These findings contribute to our understanding of how the SC processes visual inputs, a critical step in comprehending visually guided behaviors.

Introduction

The superior colliculus (SC) is a midbrain structure that integrates inputs from different sensory modalities and controls mul-

tle vision-dependent behaviors. In addition to its well known role in eye/head movements (Sparks, 1986), the mouse SC contributes to other visually evoked behaviors such as escape and/or freezing in response to a looming object (Shang et al., 2015; Wei et al., 2015) and quick suspension of locomotion (Liang et al., 2015). In fact, mice that develop without a cortex can perform visually guided memory tasks, suggesting that the SC has functions that are often attributed to the cortex (Shanks et al., 2016).

Received Nov. 28, 2016; revised July 4, 2017; accepted July 12, 2017.

Author contributions: S.I., D.A.F., and A.M.L. designed research; S.I. performed research; S.I. contributed unpublished reagents/analytic tools; S.I. analyzed data; S.I., D.A.F., and A.M.L. wrote the paper.

This work was supported by Brain Research Seed Funding provided by UCSC and the National Institutes of Health (Grant NEI R01EY022117 to D.A.F. and National Eye Institute Grant R21EY026758 to D.A.F. and A.M.L.). We thank Michael Stryker for training on the electrophysiology experiments and his very helpful comments on the manuscript; Sotiris Masmanidis for providing us with the silicon probes; Forest Martinez-McKinney and Serguei Kachiguin for their technical contributions to the silicon probe system; Jeremiah Tsyaporin for taking an image of neural tissues and the training of mice; and Jena Yamada, Anahit Hovhannissyan, Corinne Beier, and Sydney Weiser for helpful comments on the manuscript.

The authors declare no competing financial interests.

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DOI:10.1523/JNEUROSCI.3689-16.2017

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The SC in all animals is organized into several laminae, each of which has distinct inputs and outputs (May, 2006). The stratum opticum (SO) contains retinal and cortical axons and approximately divides the SC into two parts: (1) the SO and above are the superficial SC (sSC), which receives inputs from the retina and the primary visual cortex (V1) and responds to visual stimuli and (2) below the SO is the deep SC (dSC), which contains multimodal cells that respond to visual, auditory, and/or somatosensory stimuli. The response properties of the mouse sSC cells have been characterized and show selectivity to stimulus features such as orientation or direction (Wang et al., 2010; Inayat et al., 2015). Moreover, Gale and Murphy (2014) identified four cell types in the sSC that differ in their response properties and axonal targets, suggesting that different visual features are segregated into different SC cell types.

Compared with the sSC, much less is known about the visual response properties of the dSC cells. In primates, the only reported difference between the sSC and the dSC is the size of preferred stimuli (Humphrey, 1968). In mice, the only electrophysiological studies of the dSC are the original studies conducted by Dräger and Hubel (1975a, b, 1976) and Dräger (1975), which reported that dSC cells have large RFs, are more frequently direction-selective (DS), and can be multimodal. These early results provide important hints of the dSC cell properties, but the number of recorded neurons was small (<100) and the orientation-selective (OS)/DS properties were poorly quantified. In addition, there are other response properties that give clues to a cell's function that have not been explored in the dSC. These include two distinct types of nonlinear spatial summation properties: the cortical complex-cell-like nonlinearity measured by drifting sinusoidal gratings (C-like nonlinearity; Skottun et al., 1991) and the retinal Y-cell-like nonlinearity measured by contrast reversing gratings (Y-like nonlinearity; Hochstein and Shapley, 1976).

To fill this knowledge gap, we have used large-scale silicon probe neural recordings to examine the visual response properties of the SC cells of head-fixed, alert mice watching a variety of visual stimuli. We also developed a model-based analysis that provides a thorough parameterization of the response properties and simple significance tests. Using a dataset with 1445 identified neurons from both the sSC and the dSC, we discovered several novel visual response properties, differences between the visual functional organization of the sSC and the dSC, and three different types of modulation in the visual response properties of SC neurons due to locomotion of the mouse. Together, our large-scale recordings provide an important step to gaining a comprehensive analysis of the visual response properties of cells in the mouse SC with the goal of understanding how this area contributes to visually guided behaviors.

Materials and Methods

Procedures. All procedures were performed in accordance with the University of California, Santa Cruz (UCSC) Institutional Animal Care and Use Committee. We recorded the activity of the collicular neurons of head-fixed, alert mice using 128-electrode or 256-electrode multishank silicon probes. Our experimental procedures were described previously (Shanks et al., 2016) and are detailed below.

Experimental design and statistical analysis. The statistical methods used in this study are described in detail in the following subsections; here we give a brief summary. We used: (1) a χ^2 minimization fit and a χ^2 test for evaluating the goodness-of-fit; (2) an unbinned maximum-likelihood estimation; (3) ANOVA for testing the significance of the fit parameters; (4) the Kolmogorov–Smirnov (KS) test for comparing the distributions of continuous variables; and (5) the Pearson's χ^2 test for

comparing categorical distributions. The Bonferroni correction was applied when multiple comparisons were made.

Blind analysis. Blind analysis is an effective method for reducing the false-positive reporting of results (Klein and Roodman, 2005; MacCoun and Perlmutter, 2015). We looked for specific and/or significant features in a randomly subsampled set of 10 of the 20 recordings. After specifying the features for further investigation and freezing the individual analyses, we unblinded the other 10 recordings to check whether the significance of the results was reproduced. All of the results shown here passed the significance test before and after unblinding unless noted otherwise. We report the results of the combined data.

Animal preparation and experimental setup. Two- to five-month-old C57BL/6 male mice were used in this study. Before the recording date, we implanted a stainless steel head plate on the mouse's skull, which allowed us to fix the mouse's head to the recording rig. Mice were trained to behave freely on a spherical floating ball treadmill (Dombeck et al., 2007; Niell and Stryker, 2010) for at least four training sessions (30 min per training session, each session on a different day). On the day of the recording, the mouse was anesthetized with isoflurane (3% induction, 1.5–2% maintenance) and a craniotomy (~1.5 mm diameter) was performed in the left hemisphere at a site that was 0.6 mm lateral from the midline and 3.7 mm posterior from the bregma. During the surgery, the mouse eyes were covered with artificial tears ointment (Rugby), which was removed before recovery from anesthesia with a wet cotton swab. The incision was covered with 2% low-melting-point agarose in saline to keep the exposed brain tissue from drying. The probe was inserted through the cortex toward the SC; the end of the probe was located 1800–2300 μ m below the cortical surface. Recordings were started 30 min after probe insertion. We recorded from each mouse only once with a single probe insertion.

During the recording sessions, the mouse was allowed to behave freely on the treadmill. The movement of the treadmill was recorded by two optical computer mice attached to the side and the back of the treadmill at a sampling rate of 60 Hz.

A visual stimulus monitor (Samsung S29E790C; 67.3 cm $\times$ 23.4 cm active display size; mean luminance: 32 cd/cm²; refresh rate: 60 Hz; gamma corrected) was placed 25 cm away from the right side of the mouse (see Fig. 1A). Activity in the SC was identified by displaying either an ON (white) or OFF (black) flashing spot on the monitor and determining whether visual responses were localized to a restricted area within the visual field. The monitor position and angles were adjusted so that the RF was at the center of the monitor and the monitor plane was perpendicular to the line of sight.

Silicon probe electrophysiology. The silicon probe recording was performed as described in our previous report (Shanks et al., 2016) with an important modification. Instead of 64- and 128-electrode two-shank silicon probes, we used four-shank probes with 128 electrodes and 256 electrodes (see Fig. 1B). All of the probes used for our recordings were kindly provided by Prof. Masmanidis at University of California–Los Angeles (Du et al., 2011; Shobe et al., 2015). A small amount of DiI was put on the back of the probe shanks. The locations of the shanks were reconstructed histologically after recordings using the DiI traces (see Fig. 1C). A ground wire was placed on the skull near the craniotomy site by submerging it into the agarose. The voltage traces from all of the electrodes were amplified and sampled at 20 kHz using an RHD2000 256-channel recording and data acquisition system (Intan Technologies). The visual stimuli were synchronized to the recorded neuronal activity via electrical pulses sent from the visual stimulation computer to the data acquisition board.

Visual stimuli. Four different visual stimuli were used to evaluate the visual responses of the SC neurons. The first were individual 10° diameter flashing circular spots on a 10 $\times$ 7 grid with 10° spacing. The 500 ms flashes were either ON (white) or OFF (black) on a gray background at mean luminance and a 500 ms gray screen was inserted after each stimulus presentation. The stimulus contrast and the location on the grid were chosen in a random order. Each pattern (every combination of the grid locations and the contrasts) was repeated 12 times (Wang et al., 2010). The second type of stimuli were drifting sinusoidal gratings; the parameters were the same as those used previously (Niell and Stryker,

2008). The sinusoidal gratings were moving in 12 different directions (30° spacing) with 6 spatial frequencies in the range 0.01–0.32 cycles/degree (cpd) with geometric steps. The temporal frequency and duration were 2 Hz and 1.5 s, respectively. We also presented full-field flickering (0 cpd) and gray screen. Each pattern was presented in a random order and repeated 12 times. The third type of stimuli were contrast-reversing sinusoidal gratings with 7 spatial frequencies in the range 0.02–1.28 cpd with geometric steps. The amplitudes were sinusoidally modulated at 4 Hz. Two different spatial phases (0° and 90°) and four different orientations (0°, 45°, 90°, and 135°) were used for each spatial frequency. Each pattern was presented in a random order and repeated 10 times. The final type of stimulus was a contrast-modulated noise movie adapted from Niell and Stryker (2008). Briefly, a Gaussian white noise movie was generated in the Fourier domain with the spatial frequency spectrum set to $A(f) = 1/(f + f_c)$, with $f_c = 0.05$ cpd and a sharp low-pass cutoff at 0.12 cpd. The temporal spectrum was flat with a sharp low-pass cutoff at 4 Hz. This frequency domain movie was then transformed into the temporal domain and a 0.1 Hz sinusoidal contrast modulation was added. The movie was originally generated at 128×64 pixels and expanded to the size of the stimulus monitor with smooth interpolations between pixels.

In addition to these stimuli, a contrast-alternating checkerboard stimulus (0.04 cpd square wave alternating at 0.5 Hz) was used to collect the local field potentials (LFPs), which were used to estimate the surface location of the SC (see the “Electrophysiological identification of the SC surface” section).

Some analyses require visual stimuli that were used in only a subset of experiments. In those cases, the number of neurons used for the analysis is indicated in the corresponding Results section.

Spike-sorting and extraction of the local field potential. For spike-sorting and local field potential analysis, we used custom-designed software as described previously (Litke et al., 2004; Shanks et al., 2016). A level 5 discrete wavelet filter (Witltschko et al., 2008) (cutoff frequency ~313 Hz) was applied to the recorded data. The high-pass part was used for single-unit identification after motion artifact removal and the low-pass part was used for the LFP analysis. The average motion artifact shape was estimated as a function of time by averaging the signals of all of the recording channels. The estimated artifact was then subtracted from each channel signal with a multiplicative factor that minimized the root mean square of the channel’s signal.

Individual neuron identification was based on a previously developed method (Litke et al., 2004). The waveform of a spike detected on a “seed” electrode was combined with the time-correlated waveforms on the neighboring electrodes. Principal component analysis was used to extract the most significant variables for spike sorting from the waveform measurements. These variables were then clustered using the expectation maximization algorithm. After the first fit, the number of clusters was reduced one by one and refit until it no longer lowered the Bayesian information criterion (Fraley and Raftery, 1998). To remove duplicates and bad clusters, we used the contamination index (>0.3 ; Litke et al., 2004), isolation distance (<20 ; Schmitzer-Torbert et al., 2005), L-ratio (>0.1 ; Schmitzer-Torbert et al., 2005), spike-correlation (>0.25 ; Litke et al., 2004), similarity of electrophysiological images (>0.95 inner product), and the firing rate (requires >0.1 Hz in the first and last 5 min segments of the visual stimulus). When responses to multiple visual stimuli were compared, we required the firing rate criterion to be satisfied for all of the visual stimuli. Once the neurons were identified, the positions of the neurons were estimated by fitting a 2D circular Gaussian to the spatial extent of the average spike amplitudes (i.e., electrophysiological images; for details of the neurons, see Litke et al., 2004).

Electrophysiological identification of the SC surface. We identified the surface of the SC using the LFPs recorded on each electrode. A flashing checkerboard stimulus generates a strong LFP in the area where the sSC is located (Zhao et al., 2014). We used the following steps to identify the surface line of the SC. Step (1): on a vertically aligned column of electrodes, we average the evoked LFPs induced by the checkerboard contrast reversal to get the LFP as a function of the depth and the time after the reversal (see Fig. 2A). Step (2): Choose the time when the LFP has the maximum negative amplitude and, at this specific time, obtain the LFP amplitude as a function of depth (see Fig. 2B). Step (3): Choose the depth

where the LFP is half-maximum and closer to the top of the shank. This point is defined as the surface position for the column. Step (4) Repeat Steps (1) through (3) for all available electrode columns (8 columns for 128-electrode probes; 12 columns for 256-electrode probes), and obtain a surface point for each column. Step (5) Fit a line to the identified surface points using the method of least squares. This electrophysiologically estimated SC surface agrees well with the actual surface of the SC (see Fig. 1C, dashed white line). The perpendicular distance from this line defines the depth of the cells in the SC.

This procedure will produce a similar result to that of the current source density (CSD) analysis. The CSD analysis, based on the second-order derivative of the LFP with respect to depth, is an established method for identifying physiological landmarks of anatomical structures (Niell and Stryker, 2008; Zhao et al., 2014). The zero-crossing point of the CSD can be used to identify the surface of the SC. However, if the LFP amplitude is estimated as a Gaussian function of depth (with mean μ and SD σ), which is a reasonable model given the shape shown in Figure 2B, then the zero-crossing point of the CSD, $\mu \pm \sigma$ (with $\sigma \sim 100 \mu\text{m}$), is not very different from the half-maximum point, $\mu \pm 1.18 \sigma$. In addition, the half-maximum point has the following advantages: (1) resilience to the asymmetry of the LFP shape; (2) robustness to the outlier amplitudes outside of the region of interest; and (3) methodological/computational simplicity. Therefore, we decided to use the half-maximum point method instead of the CSD method.

We defined the border between the sSC and dSC as $400 \mu\text{m}$ in depth measured perpendicularly from the surface. This is based on the change of the physiological properties in our own data, as well as the fact that a border line between the SO and the stratum griseum intermedium was drawn consistently at $\sim 400 \mu\text{m}$ in other studies (Phongphanphane et al., 2008; Hong et al., 2011; Zhao et al., 2014). We do not distinguish any further sublamina of the SC; our dSC includes areas that are sometimes referred to as the intermediate and/or deep SC.

Spontaneous firing rate. We define the spontaneous firing rate of an individual neuron as the firing rate while the mouse watches a gray screen. An accurate measurement of the spontaneous firing rate is important because the significance and sign of the neuronal response is evaluated relative to this rate. We tried two different methods for evaluating the spontaneous firing rate. Our drifting grating stimulus consists of 1.5 s stimulus periods and 0.5 s gray screen intervals. In the 12 stimulus periods, we displayed a blank gray screen pattern to evaluate the spontaneous firing rates ($1.5 \text{ s} \times 12 = 18 \text{ s}$ total time for evaluation). In addition, we also evaluated the spontaneous firing rates during the intervals after removing the first 0.2 s, which is affected by switching from the stimulus to the gray screen ($0.3 \text{ s} \times 887 = 266.1 \text{ s}$). The two spontaneous firing rates did not differ significantly ($p > 0.01$) for most neurons (92%). Therefore, we used the spontaneous firing rates evaluated by the intervals because they are more precise.

Modeling of the orientation/direction selectivity with a χ^2 fit. We used χ^2 minimization to fit our model functions to the firing rate of a cell in response to stimuli with different directions (direction tuning curve, DTC). A similar approach had been taken in a previous study to estimate the best model function for the orientation tuning curve (Swindale, 1998). The χ^2 is defined as follows:

$$\chi^2 = \sum_{i=1}^{12} \frac{(R_{\text{obs}}^i - R_{\text{model}}^i)^2}{(\sigma_{\text{obs}}^i)^2} \quad (1)$$

where the sum is over all of the 12 directions i , R_{obs}^i is the observed average firing rate, R_{model}^i is the estimated firing rate from the model functions that are defined below, and σ_{obs}^i is the SEM of the observed firing rate. The preferred spatial frequency of a neuron was chosen as the frequency that caused the most significant firing rate change from the spontaneous rate, either positive or negative.

The Python SciPy “curve_fit” function was used for fitting functions. (To have proper error propagations, the version needs to be ≥ 0.17 .) A goodness-of-fit test was done for each fit with the evaluation of the p -value. The p -value distribution is a useful indicator of how well the model fits the data (see Fig. 3E).

Table 1. Calculation of the OS/DS properties by fit parameters

	Gaussian	Sinusoid
Evoked baseline firing rate	A	A
OS amplitude	(B + C)/2	C
DS amplitude	(B – C)	2B
Preferred orientation/direction	E, E + π	D, D + π
Tuning width	D	N/A
Maximum firing rate	A + max(B, C)	max(A + B + C, A – B ² /4C)
Minimum firing rate	A + min(B, C)	min(A – B + C, A – B ² /4C)

Two model functions were used for the fit: wrapped Gaussians and sinusoid.

For wrapped Gaussians:

$$R(x) = A + \sum_{n=-\infty}^{\infty} \left(B \exp\left(-\frac{(x - E + 2n\pi)^2}{2D^2}\right) + C \exp\left(-\frac{(x - E + (2n + 1)\pi)^2}{2D^2}\right) \right) \quad (2)$$

where $R(x)$ is a periodic function: $R(x + 2\pi) = R(x)$. In the actual implementation, the range of n is set to -3 to 3 , which serves as a practical approximation of this function for $0 < x < 2\pi$.

As previously reported, the Gaussian fit does not always converge if the parameters are unbounded (Mazurek et al., 2014). We introduced fit parameter boundaries that are similar to Mazurek et al. (2014) as follows:

1. $0 < A < \max(\text{DTC})$ (to avoid blowup of the baseline, which happens when the width is large).
2. $(\text{bin width})/2 < D < \pi/2$ (min: to avoid overfitting by shrinking Gaussians; max: to avoid excessive overlapping of the adjacent Gaussians).
3. $-4\pi < E < 4\pi$ (to avoid E getting out of the defined function)

For sinusoid:

$$R(x) = A + B \cos(x - D) + C \cos^2(x - D) \quad (3)$$

There are no parameter restrictions for the sinusoidal model.

The fit parameters were evaluated with an error matrix (Hessian matrix). As described previously (Mazurek et al., 2014), the error is not trustworthy when the fit parameter is at the manually set boundaries; however, even if some parameters are at the boundaries, the errors of the other parameters are still valid. We used the error values only when the fit parameters were not at their boundaries.

To compare the results of the fits from these two different fit functions, we calculated various OS/DS properties from the fit parameters (Table 1). When arithmetic calculations were performed on the parameters, the errors were appropriately propagated using both the variance and the covariance of the parameters. A cell with a significant (positive or negative) DS amplitude ($p < 0.001$) was classified as a DS cell and a non-DS cell with a significant OS amplitude ($p < 0.001$) was classified as an OS cell. We used a significance threshold at $p = 0.001$ to reduce the fraction of false-positive OS/DS cells in the subsequent analysis. For example, assuming that 20% of all of the cells are OS cells, applying $p = 0.01$ and $p = 0.001$ thresholds will result in having $\sim 4\%$ and $\sim 0.4\%$ of the candidate OS cells, respectively, be false-positives.

The maximum and minimum firing rates determine whether a neuron had a positive response, a negative response, or both. If the maximum/minimum firing rate is significantly higher/lower ($p < 0.01$) than the spontaneous firing rate, then the neuron has a positive/negative response, respectively.

This model-based method resolves the issues of the traditional methods used by others, which subtract the spontaneous firing rate from the response (Niell and Stryker, 2008; Wang et al., 2010; Inayat et al., 2015). The treatment of the negative firing rate caused by this subtraction is not explicitly explained in these studies. If the negative part is truncated, then it loses information of the negative part; if the negative part is left as negative, then the definition of the preferred angle, the weighted sum of the phase, becomes ill defined. In addition, the resulting parameters

(orientation/direction selectivity index; OSI/DSI) were not checked for significance. Mazurek et al. (2014) introduced a method of significance tests, but the relation with the spontaneous firing rate was not discussed in their study. Our model-based method provides a proper treatment of both the spontaneous firing rate and the significance tests.

Goodness of fit and goodness of the model. The goodness of fit was evaluated for each fit. If the fit is good, then the minimized χ^2 values should follow the χ^2 distribution. The two models have a different number of parameters (five for the Gaussian fit and four for the sinusoidal fit) and therefore a different number of degrees of freedom (seven and eight, respectively). To cross-compare the χ^2 distributions with different numbers of degrees of freedom, we calculated the goodness of fit p -values using the cumulative probability distribution function of the χ^2 distribution. High p -values indicate overfitting or overestimated errors; low p -values indicate too few parameters, an incorrect model, or underestimated errors.

Modeling of flashing-spot responses with an unbinned maximum-likelihood fit. A flashing spot can elicit spikes for most of the sSC cells (Wang et al., 2010). We characterized the spatial and temporal properties of the RFs of the SC cells by parameterizing the response to the spots shown at various locations of the 10×7 grid on the screen. However, the number of spikes elicited by a flashing spot is typically smaller than those elicited by a full-field stimulus and the responses can be short in time (some transient responses have ~ 50 ms temporal widths). This combination makes the analysis challenging. Traditional time-bin-based methods will generate many bins with no spikes, which cause failure of the function fit. Increasing the bin size sacrifices the temporal resolution. To resolve this issue, we used unbinned maximum-likelihood estimation (UMLE) to parameterize the response. The UMLE uses the individual timing of all of the spikes collected from every grid location and eliminates the need for time bins. This method has been commonly used for experimental data analyses in the physical sciences and is known to work well for data with a small number of events (Lyons, 1989; Cowan, 1998).

The fit is performed by maximizing the likelihood function. The likelihood function is the normalized probability distribution for spike generation, which includes both stimulus-evoked and spontaneous spikes. We defined the likelihood function of an individual spike as follows:

$$L(x, y, t) = aL_s(x, y)L_t(t) + (1 - a)F_s \quad (4)$$

where x is the azimuth, y is the elevation, t is the spike time, L_s is the spatial part of the likelihood function, L_t is the temporal part of the likelihood function, F_s is the contribution of the spontaneous firing, and a is the fraction of the signal component. Note that F_s is a constant, which is also normalized across space and time ($\iint F_s dx dy dt = 1$). The spatial part is defined by a normalized 2D Gaussian function as follows:

$$L_s(x, y) = \frac{1}{2\pi\sigma_x\sigma_y} \exp\left(-\frac{x'^2}{2\sigma_x'^2} - \frac{y'^2}{2\sigma_y'^2}\right), \quad (5)$$

$$x' = (x - \mu_x) \cos \theta - (y - \mu_y) \sin \theta,$$

$$y' = (x - \mu_x) \sin \theta + (y - \mu_y) \cos \theta$$

where μ is the center of the Gaussian, σ is the width of the Gaussian, θ is the tilt of the Gaussian, x' and y' are the translated and rotated coordinates about the center of the Gaussian, respectively. The temporal part is defined by an asymmetric Gaussian with a constant bias on the right as follows:

$$L_t(t) = \begin{cases} A \exp\left(-\frac{(t - t_{peak})^2}{2\sigma_l^2}\right), & t < t_{peak} \\ (A - b) \exp\left(-\frac{(t - t_{peak})^2}{2\sigma_r^2}\right) + b, & t \geq t_{peak} \end{cases} \quad (6)$$

where A is the normalization factor, t_{peak} is the peak response time, σ_l is the Gaussian width on the left side, σ_r is the Gaussian width on the right side, and b is the bias on the right that expresses the sustained component. The normalization factor A is set to satisfy the following equation:

Table 2. Boundaries of the fit parameters for flashing-spot responses

	Minimum	Maximum
Signal component (α)	0 (–1 when evaluating negative response)	1
Spatial ellipse azimuthal center (μ_x)	Left edge of the monitor	Right edge of the monitor
Spatial ellipse elevational center (μ_y)	Bottom edge of the monitor	Top edge of the monitor
Spatial ellipse width 1 (σ_x)	5°	100°
Spatial ellipse width 2 (σ_y)	5°	100°
Spatial ellipse tilt (θ)	–360°	360°
Peak response time (t_{peak})	0.02 s	0.4 s
Temporal width (left) (σ_l)	0.01 s	0.25 s
Temporal width (right) (σ_r)	0.01 s	0.25 s
Sustained response (b)	0/s	10/s

$$\int_0^{0.5} L_t(t) dt = 1$$

The integral is over the 0.5 s stimulus duration.

The overall likelihood value for each cell is calculated as a product of the likelihoods for the collection of individual spikes produced by the neuron. We fit the spot responses using these 10 parameters: α , σ_x , σ_y , μ_x , μ_y , θ , t_{peak} , σ_l , σ_r , and b . These parameters are bounded to an area for numerical stability; the boundaries are summarized in Table 2. Note that b has a unit of 1/s because L_t is a normalized probability distribution function of time. This should not be interpreted as the spike rate. The parameter α is allowed to have a negative value only when we evaluated the negative change of the firing rate.

Because of the bias of the firing rate on the right side (b), it is difficult to extract the duration of the response from these parameters. To have an idea of the temporal center of the response, we calculated the temporal median (t_{med}), which satisfies the following equation:

$$\int_0^{t_{med}} L_t(t) dt = 0.5 \quad (8)$$

This is the time that divides the temporal likelihood function equally and gives the temporal center of the response.

For cells with many spikes, the likelihood value can be a very small number. To avoid a floating-point underflow, the negative log-likelihood function was used for the actual implementation. The minimization was performed using FMINUIT (binding of MINUIT; James and Roos, 1975) for Matlab (http://www.fis.unipr.it/~giuseppe.allodi/Fminuit/Fminuit_intro.html).

Analysis of contrast reversing gratings. The contrast reversing gratings is a spatial sinusoidal pattern that changes contrast sinusoidally over time. By using a wide range of spatial frequencies, including frequencies that exceed the cells' RF spatial resolution limit, it is possible to characterize the Y-like nonlinear spatial summation property (Hochstein and Shapley, 1976; Petrusca et al., 2007). Responses at the 4 Hz stimulus temporal frequency (F_1) and the second harmonic frequency (F_2) were characterized as a function of spatial frequency using a method described previously (Hochstein and Shapley, 1976; Petrusca et al., 2007). Namely, for each spatial frequency, the phase dependence was taken into account by taking the maximum F_1 value, and the mean F_2 value, over the two spatial phases.

Neurons are considered to have Y-like nonlinearity of spatial summation when they have a significant response in the F_1 component at the lowest spatial frequency (0.02 cpd, $p < 0.01$), and have a significantly stronger F_2 response in at least one of the higher frequencies ($p < 0.01$, with the Bonferroni correction). In a primate retina study, the nonlinearity index (maximum value of F_2/F_1 ratio over the spatial frequencies) has been used (Petrusca et al., 2007). The nonlinearity index is a good indicator of the Y-like cells in the retina, where most of the neurons are either ON cells or OFF cells. In the SC, many of the cells are ON–OFF cells (Wang et al., 2010), which have a strong F_2 component even in a low

spatial frequency range. The method used in the present study ensures that a cell has the proper characteristics of a Y-like cell: a strong F_1 component at a low spatial frequency and a strong F_2 component at a high spatial frequency.

Analysis of the contrast-modulated noise movie. The contrast-modulated noise movie is an effective stimulus to find out how cells respond to contrast. To characterize the contrast response of the cells, we used a function fit method similar to the analysis of the orientation selectivity. We considered the zero-contrast timings as the starting times of the stimulus and constructed a poststimulus time histogram (PSTH) for each neuron. The same sinusoidal function (Eq. 3), with a 10 s temporal period, was fit to the PSTH (χ^2 fit using SEM of the PSTH) to get the amplitude of the linear response (model parameter B). Among the cells with a significant linear response, those with a maximum firing rate at 4–6 s (when the contrast is maximum) are considered as stimulated-by-contrast (StC) cells; those with a maximum firing rate at 0–1 s and 9–10 s are considered as suppressed-by-contrast (SuC) cells. “Other” cells include those with a significant response with a maximum firing rate timing that does not meet the above criteria. These cells have a nonlinear relation between the stimulus contrast and the firing rate. The “nonresponsive” cells include those that did not have a significant response.

Analysis of the modulation by locomotion. It is known that the activity of V1 cells is modulated by locomotion (Niell and Stryker, 2010; Fu et al., 2014). To evaluate how locomotion modulates the SC cell activity, we separated the periods when the mouse is stationary from the periods when it is in locomotion and measured the firing rate change in response to the drifting sinusoidal gratings between these two behavioral states. The speed of locomotion is sampled at 60 Hz by two optical mice attached to the spherical treadmill. The speed measurement is convolved with a Gaussian filter with 250 ms full width at half maximum and resampled at 20 Hz. We used a 1 cm/s threshold value to distinguish the stationary state from the running state, as used previously (Niell and Stryker, 2010; Fu et al., 2014). Each stimulus presentation is considered as a stationary trial if the mouse was stationary for >80% of the period; it is considered as a running trial if the mouse was running for >80% of the period. If neither was satisfied, the trial was not used. To have a sufficient number of both stationary and running trials, we only used experiments in which the mouse spent 20–80% of the total time running.

The effect of the modulation can be evaluated both as a function of the spatial frequency and the direction of the moving gratings. However, because we only had 12 trials for each stimulus pattern, in many cases, we did not have a sufficient number of trials of different behavioral states in all 12 directions. Therefore, we combined the data from all of the directions and characterized the effect as a function of the spatial frequency.

To model the effect of modulation, we took an approach that has been used to describe how population activity modulates macaque V1 neurons (Arandia-Romero et al., 2016). This model estimates the additive and multiplicative components of the modulation. In our implementation, the additive component is the constant shift of the firing rate between the two behavioral states (stationary and locomotion); the multiplicative component is the ratio of the stimulus-evoked firing rate between the two states. First, we divided the firing rate of the stationary (stat) trials F_{stat} into the evoked firing rate and the spontaneous firing rate as follows:

$$F_{stat}(f) = E_{stat}(f) + S_{stat} \quad (9)$$

where E is the evoked firing rate, S is the spontaneous firing rate, and f is the spatial frequency. Then, we modeled the firing rate during the locomotion (loco) periods using a gain modulation (a) and a baseline shift (b) as follows:

$$F_{model}(f) = E_{model}(f) + S_{model} = a E_{stat}(f) + S_{stat} + b \quad (10)$$

To fit a and b , we minimize the χ^2 value defined as follows:

$$\chi_{AM}^2 = \frac{(S_{loco} - S_{model})^2}{\sigma_S^2} + \sum_f \frac{(F_{loco}(f) - F_{model}(f))^2}{\sigma_F^2(f)} \quad (11)$$

where σ_S and σ_F are the errors of the spontaneous and total firing rates, S_{loco} and F_{loco} , respectively. Because we have errors of the model estimate

Figure 1. Experimental setup. **A**, Drawing of the recording setup. During the recording, the head-fixed mouse is alert on a floating spherical treadmill and watches a variety of visual stimuli. **B**, Schematic of a 256-electrode, four-shank silicon probe (200 μm shank pitch, each shank is 86 μm wide and 23 μm thick; three columns of electrodes on each shank, 20 μm column pitch; 21–22 electrodes on each column, 50 μm electrode pitch; 1050 μm active length, 640 μm lateral extent; used for 15 of 20 recordings). The background image is a sagittal section of the SC taken from the Allen Mouse Brain Atlas (Lein et al., 2007) with labels modified to match the terms in the present study. Scale bar, 400 μm . **C**, Photograph of a parasagittal section of the SC after recording indicating the probe location as marked by Dil. The dotted cyan lines indicate the recording shank locations reconstructed by the Dil traces (red channel). A dashed white line indicates the electrophysiologically estimated surface of the SC, which agrees well with the actual surface. A dashed yellow line indicates the estimated border between the sSC and the dSC, which is 400 μm below the estimated surface of the SC. Scale bar, 400 μm .

Figure 2. SC surface estimation and firing rate distribution. **A**, Image of the average LFP evoked by a checkerboard stimulus. The image represents data collected from electrodes in one 22-electrode column. The color indicates the LFP signal amplitude in microvolts. Vertical axis gives the depth from the top electrode. Horizontal axis gives the time after the contrast reversal of the checkerboard stimulus. Black dashed line indicates the time when the amplitude of the LFP response is maximized. **B**, LFP signal amplitude as a function of depth at the time indicated by the black dashed line in **A**. The red dashed line indicates the estimated surface of the SC for the electrode column, where the LFP signal amplitude is 1/2 of its maximum amplitude. Error bars indicate SEM over 600 trials. **C**, Number of identified neurons as a function of the depth (blue) and the number of neurons per electrode as a function of the depth (red). The neurons with a negative depth are cortical neurons. The gap between the cortex and the SC right above zero (depth ~ -100 μm) confirms that the depth of the SC surface is estimated well. **D**, Distribution of the spontaneous firing rate of the sSC (blue) and dSC (red) cells. dSC cells are more spontaneously active.

that are inherited from the stationary period measurement, these errors are combined errors of the locomotion period and the model estimate. For each neuron, the gain modulation is significant if a is significantly ($p < 0.01$) different from 1; the baseline shift is significant if b is significantly ($p < 0.01$) different from 0.

Results

Large-scale silicon probe recordings create a large dataset of visually responsive neurons throughout the depth of the SC
We recorded from the SC of alert mice on a spherical treadmill (Fig. 1A) using large-scale silicon probes (Fig. 1B). The active

length of the probe is long enough to record from both the sSC and the dSC simultaneously. The surface location of the SC was estimated by the LFPs induced by the alternating checkerboard stimulus (Fig. 2A,B; see “Electrophysiological identification of the SC surface” section in Materials and Methods), and the neurons were divided into the sSC (depth < 400 μm) and the dSC (depth ≥ 400 μm) neurons according to their measured locations. The high spatial density, 128- and 256-electrode silicon probe recordings identified a total of 1445 SC neurons (687 superficial, 758 deep) in 20 mice, with an average number of 0.43 ± 0.03 isolated single neurons per electrode in the SC. Figure 2C shows the distribution of the cell counts and the number of cells per electrode throughout the depth of the SC. The spontaneous firing rate was 3.7 ± 0.3 Hz in the sSC and 5.6 ± 0.3 Hz in the dSC (mean $\pm$ SEM). However, because the spontaneous firing rate distributions have a long tail, the mean and the SEM in log space may better characterize these distributions (Fig. 2D). In log space, the spontaneous firing rate was $10^{-0.10 \pm 0.03}$ Hz in the sSC and $10^{0.19 \pm 0.03}$ Hz in the dSC (~ 0.8 Hz and ~ 1.6 Hz, respectively). The dSC cells have significantly higher spontaneous firing rates than the sSC cells ($p = 7 \times 10^{-10}$). Note that the dSC has a significantly larger fraction of cells with high

(> 10 Hz) spontaneous firing rate (sSC: $11.5 \pm 1.2\%$, dSC: $20.1 \pm 1.4\%$; $p = 6 \times 10^{-6}$). The high spontaneous firing rate is important for cells that respond to a stimulus with a negative firing rate change.

Characterization of orientation and direction selective cells using a novel model-based analysis

We developed a model-based fit method to quantify the visual response properties of the SC cells. OS/DS responses have been traditionally characterized by calculating preestablished

Figure 3. OS and DS cell response examples. **A**, Example drifting gratings responses of a positive OS cell. The circularly placed raster plots are the responses to the gratings moving in the corresponding directions. The gray horizontal bars indicate the beginning and end of the stimulus. The inset bar graph indicates the average response firing rate for each direction (black histogram), the spontaneous firing rate (red line), and the result of a function fit (blue solid line). Error bars indicate the SEM of 12 trials. Two polar plots to the right of the histogram indicate the polar plot representation of the response histogram. The polar plots are the DTCs of the cell without (top) and with (bottom) the spontaneous firing rate subtracted. **B**, Same set of figures as **A** but for a negative DS cell. The firing rate is significantly lower than the spontaneous rate $\sim 190^\circ$. Note that the polar plots no longer represent the correct characterization of the response property of this neuron. **C**, Response histogram of a negative OS cell. Note that the spontaneous firing rate is above the responses for all of the directions, so this neuron would be neglected with the traditional approach of spontaneous firing rate subtraction. **D**, Response histogram of a DS cell that responds both positively and negatively relative to the spontaneous firing rate. This neuron also has a particularly large range of response firing rate and the firing rate seems to saturate near its preferred direction. Such a response causes deformation in the sinusoidal or Gaussian shapes and results in a poor fit ($p = 1-2 \times 10^{-6}$). **E**, Distribution of the fit p -values (of χ^2) for all of the neurons. If the fit is consistent with the data points and their corresponding errors, then the expected distribution is flat. We can estimate the fraction of neurons that were modeled poorly by the fit by counting the number of neurons above the expected flat line. Approximately 17% of the neurons exceeded the flat line, indicating that $\sim 83\%$ of the neurons were well modeled by our fit models.

indices such as the OSI or the DSI (Gegenfurtner et al., 1996; Ringach et al., 2002; Niell and Stryker, 2008), but these have been criticized for the variations of their definitions and the lack of methods for significance tests (Mazurek et al., 2014). To overcome these deficiencies, we used a model-based parameter estimation to characterize the response properties of the SC cells. To analyze the response to the drifting gratings stimuli, we used a χ^2 minimization fit, which uses the trial-by-trial SEM of the data, calculates the error matrix of the resulting parameters, and provides a goodness-of-fit measurement, which allows us to quantify how well the data are described by the model.

The model-based method effectively characterized the OS/DS cells in the SC. We used sinusoids and Gaussians to model the DTCs (see Materials and Methods). By applying a $p < 0.001$ significance threshold for the fitted OS or DS amplitudes, as defined in Table 1, we found 302 (173 superficial, 129 deep) OS cells and 201 (135 superficial, 66 deep) DS cells. Figure 3, A–D, show examples of the raster plots and tuning curves of the OS and DS cells, including: an OS cell with a positive firing rate change (Fig.

3A), a DS cell with a negative firing rate change (Fig. 3B), an OS cell with a negative firing rate change (Fig. 3C), and a DS cell with both a positive and a negative firing rate change (Fig. 3D). All firing rate changes are evaluated relative to the spontaneous firing rate (see “Spontaneous firing rate” in Materials and Methods).

The preferred spatial frequency of the OS/DS cells was $0.01 \times 2^{2.25 \pm 0.09}$ (0.048 ± 0.003) cpd in the sSC and $0.01 \times 2^{2.47 \pm 0.10}$ ($0.056^{+0.004}_{-0.003}$) cpd in the dSC (the numbers are presented in log space because the stimulus frequencies were equally spaced in log space), showing no significant difference ($p = 0.10$). The fraction of cells that are OS/DS as a function of depth is plotted in Figure 4, A and B. In the sSC, $25.1 \pm 1.7\%$ of the cells are OS and $19.6 \pm 1.5\%$ are DS; in the dSC $17.0 \pm 1.3\%$ of the cells are OS and $8.7 \pm 1.0\%$ are DS. The area close to the surface ($<150 \mu\text{m}$) is enriched with DS cells, showing qualitative consistency with Inayat et al. (2015), but differing in the estimated abundance. Inayat et al. (2015) reported that 74% of the cells in 0–50 μm depth are DS cells, whereas we found that only $44 \pm 8\%$ of our cells are DS. This difference could be due to the

Figure 4. Positive and negative responses of the OS/DS cells. **A, B**, Fraction of cells that are OS (**A**) and DS (**B**) as a function of SC depth. The surface of the SC is enriched with DS cells. **C, D**, Scatter plots of the spontaneous firing rate versus the maximum evoked firing rate in response to the drifting gratings of the OS cells in the superficial (**C**) and deep (**D**) SC areas. The red dots indicate neurons with a significant positive response. The maximum evoked firing rate is significantly ($p < 0.01$) above the spontaneous rate. A larger fraction of cells in the sSC have positive responses. Inset shows the DTC of the cell that is indicated by the arrow in **C**. **E, F**, Same figures as in **C** and **D** but for DS cells. **G, H**, Scatter plot of the spontaneous FR versus the minimum evoked firing rate in response to the drifting gratings of the OS cells in the superficial (**G**) and deep (**H**) areas. The red dots indicate neurons with significant negative responses. The minimum evoked firing rate is significantly ($p < 0.01$) below the spontaneous rate. A larger fraction of cells in the dSC have a negative response. **I, J**, Same figures as **G** and **H** but for DS cells. **K, L**, Fractions of the cells that are OS (**K**) and DS (**L**) cells that have positive, negative, and both responses to the drifting gratings. The “positive” OS/DS cells decrease and the “negative” OS/DS cells increase in the dSC ($p < 0.001$ for all combinations).

different methods used to identify the DS cells and/or our use of a relatively high level of the significance threshold ($p < 0.001$) to call a cell DS compared with the DSI method, which does not check significance.

We found that most cells are well described by our model functions. We determined the quality of the model fit for each individual cell from the χ^2 per number of degrees of freedom (ndof) (with the expectation values $\chi^2 = \text{ndof}$ and variance = $2 \times \text{ndof}$) and by the χ^2 goodness-of-fit p -value. The overall quality of the model is evaluated by the distribution of the p -values for the set of fits for the population of neurons. If the model fits correctly represent all of the data and the corresponding errors, then the p -value distribution will be uniform between 0 and 1, as indicated by the red dashed line in Figure 3E. This is a consequence of the fact that the χ^2 values of the data obey the expected “ χ^2 probability density function.” We found that 83.3% of the cells in our dataset have p -values that are below the red line expectation and $16.7 \pm 1.0\%$ of the cells (in the first three bins; $p < 0.15$) have p -values above the red line expectation. This result

indicates that the DTCs of most of the neurons are well described by our model functions.

Cells that have negative orientation or direction-selective responses are enriched in the dSC

Our model-based analysis also determines the sign (whether the firing rate change was positive, negative, or both) of each cell’s response, as well as the range of the firing rates of cells in response to the drifting gratings. By comparing this range of the firing rate with the spontaneous firing rate, we determined whether the stimulus increased or decreased the firing rate of each cell. If the neuron’s maximum evoked firing rate is significantly ($p < 0.01$) higher than its spontaneous firing rate, then the neuron has a “positive” response; if the neuron’s minimum evoked firing rate is significantly ($p < 0.01$) lower than the spontaneous firing rate, then the neuron has a “negative” response. For example, the inset of Figure 4C is the DTC of an example OS cell with a positive response. The maximum evoked firing rate of this cell exceeds its spontaneous firing rate (red horizontal line). When we plot the

maximum evoked firing rate against the spontaneous firing rate of all of the OS cells in the sSC, the cells above the line of unity slope have positive responses (Fig. 4C). The cells with a significant positive response, as defined above, are indicated in red. We performed a similar analysis of the OS and DS cells within the sSC and the dSC (Fig. 4D–F). We also determined the relationship between the minimum evoked firing rate and the spontaneous firing rate to determine whether the neuron has a “negative” response (Fig. 4G–J). We found that most of the sSC OS/DS cells have significant positive responses (Fig. 4C,E), whereas the majority of dSC OS/DS cells have significant negative responses (Fig. 4H,J). Figure 4, K and L, summarizes the results and shows that, compared with the sSC, the fraction of the positive OS/DS cells decrease (OS: $p = 4.4 \times 10^{-10}$, DS: $p = 3.5 \times 10^{-5}$) and the fraction of the negative OS/DS cells increase (OS: $p = 6.3 \times 10^{-10}$, DS: $p = 5.5 \times 10^{-7}$) in the dSC. Most cells had either a positive or negative firing rate change and only a small fraction ($7.6 \pm 1.2\%$) of the OS/DS cells responded with both positive and negative firing rate change (the maximum firing rate was significantly above the spontaneous firing rate and the minimum firing rate was significantly below the spontaneous firing rate). The analysis of the OS/DS response sign is novel and reveals an important difference of the visual information coding between the sSC and the dSC.

Suppressed-by-contrast (SuC) cells are enriched in the dSC and show little overlap with negative OS/DS cells

SuC cells are a known example of a cell type with a decrease in firing rate in the presence of a high-contrast visual stimulus (Rodieck, 1965; Tailby et al., 2007; Niell and Stryker, 2010). Because the negative OS/DS cells have a firing rate reduction by the drifting gratings stimuli, an overlap between the SuC cells and the negative OS/DS cells might be expected. To measure the fractions of the StC cells and the SuC cells, we used the “contrast modulated noise movie” adopted from Niell and Stryker (2008) (Fig. 5A), and categorized cells into StC, SuC, other, and nonresponsive cells. “Other” cells include cells with a nonlinear response to the contrast (respond only to middle contrast or show long delays). For this analysis, we used 606 neurons from 15 mice. The fraction of the StC, SuC, and other cells as a function of depth is shown in Figure 5B. Figure 5C summarizes the fractions of cells in the sSC and dSC categories. The most recognizable change from the sSC to the dSC is the decrease of the StC cells ($71 \pm 3\%$ to $30 \pm 3\%$, $p = 3 \times 10^{-28}$) and the increase of the nonresponsive cells ($10.3 \pm 1.7\%$ to $43 \pm 3\%$, $p = 3 \times 10^{-22}$). Although the SuC cells are relatively few in both the sSC and the dSC, the fraction increased by more than a factor of two ($6.1 \pm 1.4\%$ to $16 \pm 2\%$, $p = 7 \times 10^{-5}$).

To determine whether the negative OS/DS cells are a subset of the SuC cells, we measured the overlap between these cells. A total of $76 \pm 4\%$ of the positive OS/DS cells were StC cells (chance value: $51 \pm 2\%$), whereas only $22 \pm 5\%$ of the negative OS/DS cells were SuC (chance value: $11.1 \pm 1.3\%$). Although they have more-than-chance overlap, a majority ($78 \pm 5\%$) of the negative OS/DS cells are not SuC cells. This result

Figure 5. Suppressed-by-contrast (SuC) cells. **A**, Example stimulus patterns of the contrast modulated noise movie. The contrast of the movie is sinusoidally modulated over a 10 s period. **B**, Fraction of the cells, across the depth, that are conventional StC cells, SuC cells, or cells with nonlinear response to the stimulus (“other” cells). The sSC is dominated by the StC cells. After passing $\sim 400 \mu\text{m}$ in depth, the fraction of StC cells decreases and the fraction of SuC cells increases. **C**, Fractions of cells that are StC, SuC, other (nonlinear), and nonresponsive cells in the sSC and the dSC. The nonresponsive cells are those that do not change firing rate significantly in response to a contrast modulation. Despite the lack of response to the movie, $56 \pm 4\%$ of these nonresponsive cells do respond to the drifting gratings stimuli.

suggests that the negative OS/DS cells are not simply a subset of the SuC cells and that they encode different specific visual features.

dSC contains a larger fraction of cells that have larger RFs, lower evoked firing rates, and late, short transient responses

We also determined the response of the cells to flashing-spot stimuli (Fig. 6A), which is effective at stimulating sSC cells (Wang et al., 2010). For this analysis, we used a total of 1006 neurons. The number of spikes elicited by the flashing spots are few, typically only a few per stimulus. Traditional fitting methods that use time bins fail because many bins get zero spikes (of course, the time width of the bins can be increased, but this sacrifices temporal resolution). To resolve this issue, we used UMLE, which eliminates the necessity of time bins. UMLE works well even when the number of events is limited and the data have multiple dimensions (our data have two spatial dimensions and one temporal dimension). This method has been used commonly in physics to analyze sparse data (Lyons, 1989; Cowan, 1998). We modeled the spatiotemporal flashing-spot response function with a 2D spatial Gaussian multiplied by a temporal function (an asymmetric Gaussian with different widths and a baseline firing rate after the peak; see Eq. 3–6 in Materials and Methods). Within this framework, we can extract spatial and temporal parameters simultaneously. The response to flashing spots was considered significant when the signal component (α of Eq. 3) was significantly ($p < 0.01$) different from zero. $90.5 \pm 1.3\%$ of the sSC cells and $72 \pm 2\%$ of the dSC cells had a significant response. Figure 6B shows an example spatial response of a neuron that responds to a grid of both black and white flashing-spot stimuli. The red oval indicates the one σ contour, which defines the RF area. As indicated by Wang et al. (2010), most cells responded to both black and white flashing spots ($72 \pm 2\%$ of the responsive cells). In this section, we will only report the results of the response to the black stimuli because the responses to the black and white stimuli were qualitatively the same.

The sSC cells typically have earlier t_{peak} and a sustained response over time (Fig. 6C). Conversely, the dSC cells typically have a late peak time and a narrow transient response (Fig. 6D). The figure shows the binned histogram of the spikes for visualization, but the fit was performed with unbinned spikes. We plot-

Figure 6. Parameterization of the visual response properties of the SC cells in response to flashing spots. **A**, Flashing-spot stimulus patterns and example responses. Example flashing white (left) and black (right) spots are shown. These spots were shown at the 10×7 grid locations on the screen. **B**, Firing rates of an example neuron elicited by white (bottom left) and black (bottom right) spots at each grid location are shown in grayscale. This neuron responds to both black and white spots shown at a localized area of the screen. The red oval indicates the 1σ contour of the fitted 2D Gaussian. **C**, Temporal response of an example cell from the sSC. The blue line indicates the time-binned probability density function of the spikes of the neuron. The red line is the fitted function (for the details of the fit function, see Materials and Methods). This is a typical neuron in the sSC. The response is a pooled temporal response of the single neuron from all of the grid locations. The time binning is only for visualizing the data; the fit was performed on unbinned spikes. This neuron has a relatively early response onset, slow decay, and a sustained component. Therefore, the t_{peak} and t_{med} are well separated. **D**, Temporal response of an example cell from the dSC. This is a typical neuron in the dSC. The peak time is slightly later than that of the superficial neuron shown in **C**. The dSC cell has a transient response with fast rise and fall times and no sustained component; t_{med} is close to t_{peak} . **E**, A scatter plot of the depth and the RF area. Blue dots indicate individual neurons; a red line indicates the mean and SD of the RF area at each $50\ \mu\text{m}$ depth segment. Vertical histograms on the right indicate the distribution of the RF areas of the cells in the sSC and the dSC. Green horizontal lines in the histograms indicate the average of the distributions. These distributions are significantly different (KS test, $p = 5 \times 10^{-14}$). **F**, A scatter plot of the depth and evoked FR. The distributions in the sSC and the dSC are significantly different ($p = 3 \times 10^{-8}$). **G**, A scatter plot of the depth and the t_{med} . The distributions in the sSC and the dSC are significantly different ($p = 2 \times 10^{-10}$). **H**, Scatter plot of the RF areas and evoked firing rate. The blue dots are the sSC neurons, and the red dots are the dSC neurons. **I**, Scatter plot of the RF areas and the temporal median of the response. Small RF neurons typically have sustained components and thus have the temporal median at a later time. **J**, Scatter plot of the RF areas and the t_{peak} of the response. Neurons with the large RFs have a similar peak time at ~ 0.07 s and neurons with small RFs have a more variable peak time. Note that there are many neurons that have small RFs with an earlier peak time than the large RF neurons. The vertical histograms on the right are the distributions of the peak time in the sSC and the dSC. These distributions are significantly different ($p = 9 \times 10^{-7}$). *** $p < 0.001$.

ted the extracted parameters in a scatter plot to demonstrate how these parameters change as a function of the depth. We found that the area of the RFs becomes larger in the deeper part of the SC and becomes constant after $\sim 350\ \mu\text{m}$ in depth (Fig. 6E). This result is consistent with (Dräger and Hubel, 1975a) and extends the result to the deeper area. Figure 6F shows the average evoked firing rate during the 0.5 s stimulus periods at the peak of the spatial function. The dSC cells have lower evoked firing rates compared with the sSC cells. Figure 6G shows the t_{med} , the time that equally divides the elicited spikes in the 0.5 s stimulus duration (Fig. 6C,D, brown dotted line; also see Eq. 7 in Materials and Methods). Because some cells have a sustained response that lasts for the entire duration of the stimulus, extracting the exact duration from our parameter set was difficult. Therefore, we used the temporal median as a well defined alternative to the duration of the response. As Figure 6, C and D, indicates, the late and early temporal median indicates sustained and transient response, re-

spectively. Figure 6, H and I, shows the relationship among the RF areas, the evoked FRs, and the temporal median. Cells with a larger RF tend to have a lower evoked FR and an earlier temporal median. Both the sSC cells (blue) and the dSC cells (red) have such a correlation. However, a large fraction of the sSC cells have a small RF and large temporal median and a large fraction of dSC cells have a large RF and a small temporal median. Therefore, the distributions of these response properties are significantly different between the sSC and the dSC (Fig. 6E–G, vertical histograms). Figure 6J shows the scatter plot for the t_{peak} versus the RF area. Large RF cells have stereotypical values of the peak time at ~ 0.08 s, but the cells with a small RF have a more variable response peak time. Note that there is a cluster of many sSC cells that have peak times earlier than the dSC cells by ~ 10 ms. This is consistent with the anatomy that indicates that the visual input from the retina comes first to the sSC. The distributions of the RF areas, the evoked FRs, the temporal medians, and the peak times are all

Figure 7. Examples of the negative OS/DS cells that show surround suppression and negative flashing-spot responses. **A**, Spatial RF of a cell showing surround suppression. Grayscale indicates the average firing rate elicited by a flashing black spot at each grid location. Red solid ellipse is a 1 σ contour of the Gaussian fit; the yellow dashed ellipse is a 2 σ contour of the Gaussian fit. The 2 σ contour is used to define the inside and the outside of the RF. Green vertical line on the color bar indicates the spontaneous firing rate of the cell. **B**, Temporal responses of the cell shown in **A**. Blue and red lines indicate the firing rate time distributions elicited by a black spot averaged over all of the grid locations inside and outside of the RF, respectively. The green dashed line indicates the spontaneous firing rate of the cell. The blue line shows a peak that indicates a positive firing rate change in response to flashing spots. Conversely, the red line shows a trough (indicated by a red arrow) after the appearance of the spot outside of the RF, showing that the activity of this cell is suppressed when the stimulus is outside its main RF. **C, D**, Same figures as in **A** and **B** for the same neuron but in response to a white spot instead of a black spot. The response to the white spot shows a similar structure (stimulated by a spot at the RF and suppressed by a spot off the RF) as the black spot. **E–H**: Spatial (**E, G**) and temporal (**F, H**) responses of a negative DS cell that shows a negative response to a black (**E, F**) and white (**G, H**) flashing spot. This neuron responds with a significant trough of the firing rate after the stimulus onset (red arrows).

significantly different between the sSC and the dSC (KS test, $p = 5 \times 10^{-14}$, 3×10^{-8} , 2×10^{-10} , 9×10^{-7} , respectively). In summary, the sSC is enriched with cells with small RFs, high evoked FRs, and sustained temporal responses with an early peak time, whereas the dSC is enriched with cells with relatively large RFs, low evoked FRs, and transient temporal responses with a late peak time.

Majority of the negative OS/DS cells respond positively to local flashing spots, but some cells respond negatively

In an earlier section, we said that the SuC cells show little overlap with the negative OS/DS cells. To better understand what causes these negative OS/DS responses, we characterized the flashing-spot RFs of the negative OS/DS cells in detail. We used 83 negative OS cells and 51 negative DS cells in this analysis. Because the OS and DS cells did not differ significantly in their responses, we only report combined results in this section. We extended the maximum likelihood analysis of the flashing-spot response so that it can also fit to a negative response ($\alpha < 0$). Of the 134 negative OS/DS cells, 95 cells ($71 \pm 4\%$) showed a significant positive response to black or white spots, 19 cells ($14 \pm 3\%$) showed a significant negative response, and 20 cells ($15 \pm 3\%$) showed no significant responses. This result indicates that the majority of the negative OS/DS cells are excited by a local flashing-spot stimulus.

What is the origin of the negative response of the negative OS/DS cells to a drifting grating given that many of them respond positively to the small flashing spots? A possible mechanism is the surround suppression that some sSC cells are known to exhibit (Gale and Murphy, 2014). Indeed, we found that 14 of the 95 ($15 \pm 4\%$) negative OS/DS cells with a positive spot response inside of their RFs had a suppressive response outside of their RFs (FR time course with 50 ms bins has at least one point significantly below the spontaneous FR, $p < 0.01$ using Poisson statistics and the Bonferroni correction), whereas only 11 of 183 ($6.0 \pm 1.8\%$) positive OS/DS cells with a positive spot response inside of their RFs had a suppressive response outside of their RFs. Figure

7, **A–D**, shows an example neuron with surround suppression (**A** and **B** correspond to a black flashing spot; **C** and **D** correspond to a white flashing spot). This neuron responded with a positive firing rate change when the stimulus is shown inside its RF (inside of the yellow dashed line in Fig. 7A, C) and a negative firing rate change when the stimulus is shown outside of the RF (outside of the yellow dashed line in Fig. 7A, C). The firing rates as a function of time are shown in Figure 7, **B** and **D**. The red arrows indicate where the activity of the cell is suppressed. Note that this suppression is due to a single 10-degree-diameter flashing spot outside of the RF. The suppression by a full-field stimulus would be stronger than this. The sign of the response depends on the location of the flashing spot, but not whether the spot is black or white. In this sense, the response property of these neurons is different from classic center-surround ON cells or OFF cells that flip the sign of the response depending on the contrast of the stimulus.

Conversely, Figure 7, **E–H**, shows an example neuron with a negative response to the flashing-spot stimulus within its RF (**E** and **F** correspond to a black flashing spot; **G** and **H** correspond to a white flashing spot). The red arrows indicate that the cells have a negative response inside of the RF regardless of the contrast of the stimulus. A possible mechanism that explains such a response is an inhibitory connection from a positive OS/DS cell.

Most of the dSC cells have complex-cell-like spatial summation nonlinearity

The ratio of the stimulus frequency response to the mean response, F_1/F_0 , to a drifting sinusoidal grating is a standard quantitative metric of response linearity and has been used to discriminate cortical simple cells from complex cells (Skottun et al., 1991). Here, we define the cells with $F_1/F_0 < 1$ as C-like nonlinear cells and $F_1/F_0 \geq 1$ as linear cells. This metric has been applied to mouse cortical cells (Niel and Stryker, 2008; Van den Bergh et al., 2010; Bonin et al., 2011) and sSC cells (Wang et al., 2010). We have applied it to our data and found examples of both linear cells and C-like nonlinear cells in the SC (Fig. 8A). Note that the linear cell has a rhythmic response that has the fundamental frequency (2

Figure 8. Difference of F_1/F_0 linearity between the sSC and the dSC. **A**, Typical temporal response of linear (red, $F_1/F_0 = 1.97$) and C-like nonlinear (blue, $F_1/F_0 = 0.03$) cells to drifting gratings. The linear cell responds rhythmically at the stimulus frequency (2 Hz), whereas the C-like nonlinear cell elevates the firing rate without a temporal pattern. The stimulus duration was 1.5 s. **B**, Scatter plot of the depth versus the F_1/F_0 ratio. The red and green dashed lines are upper and lower threshold values that divide linear and nonlinear cells ($F_1/F_0 > 1$ is linear). The vertical dashed line at 400 μm depth is a separation line between the sSC and the dSC. Note that F_0 can have a negative value when the evoked firing rate is below the spontaneous firing rate, resulting in a negative F_1/F_0 . **C**, Histograms of F_1/F_0 of neurons with positive and negative response. The distributions differ significantly (KS test, $p = 5 \times 10^{-30}$). Most of the negative cells have a small value of F_1/F_0 , indicating that they are C-like nonlinear. **D**, Bar graph comparing the fractions of the C-like nonlinear cells in positive- and negative-responding cells. The fraction of the cells that are C-like nonlinear is significantly smaller among the cells with positive response than the cells with negative response ($p = 7 \times 10^{-36}$). **E**, Histograms of F_1/F_0 for positively responding cells. Distribution is different between the sSC and the dSC, showing a larger fraction of C-like nonlinear cells ($F_1/F_0 < 1$) in the dSC (KS test, $p = 1.8 \times 10^{-6}$). **F**, Bar graph comparing the fraction of the C-like nonlinear cells in the sSC and dSC. The dSC has a significantly larger fraction of the C-like nonlinear cells than the sSC ($p = 1.9 \times 10^{-20}$). *** $p < 0.001$.

Figure 9. Y-like nonlinear cells appear only in the sSC. **A**, An example frame of a contrast reversing grating. Instead of moving across the screen, the contrast of the pattern changes sinusoidally at 4 Hz. **B**, F_1 (4 Hz) and F_2 (8 Hz) Fourier components of the firing rate time distribution as a function of spatial frequency. This cell shows an X-like response, which has a strong F_1 component. **C**, Time course of the response of the cell shown in **B**. Response is taken at the spatial frequency indicated by the black dotted line in **B**. In one stimulus cycle (250 ms), the cell shows only one peak. **D**, Figure corresponding to **B** but for a Y-like cell. Although the F_1 component is dominant at low frequency, the F_2 component increases at high frequency and exceeds the F_1 component. **E**, Time course of the response of the cell used for **D**. Note that there are two peaks in one stimulus cycle. **F**, Fractions of the Y-like cells found in the sSC and the dSC. Almost all (42 of 43) of the Y-like cells were found in the sSC. *** $p < 0.001$.

Hz) of the drifting gratings, whereas the C-like nonlinear cell elevates its firing rate regardless of the phase of the stimulus. Figure 8B shows the value of F_1/F_0 of all of the responsive cells plotted against the depth. The distribution of F_1/F_0 changes across the depth and most cells with a linear response (above the red dashed line or below the green dashed line) appear in the sSC. Note that the negative values indicate cells with a negative mean

response ($F_0 < 0$). To clarify the differences of the cells with a positive response to those with a negative response, we plotted the two distributions of the F_1/F_0 values as histograms (Fig. 8C). Figure 8D is the quantification of the fraction of the cells that are C-like nonlinear given the response sign of the cell. Almost all of the cells with a negative OS/DS response are C-like nonlinear and this fraction is significantly different from the cells with a positive response. When we evaluated the differences between the sSC and the dSC, we found that the dSC is enriched with negative OS/DS cells, which also means that they are enriched with C-like nonlinear cells. To compare the sSC and the dSC without the bias due to the sign of the response, we plotted the distribution of the F_1/F_0 values only for positive OS/DS cells (Fig. 8E). Even among the positive OS/DS cells, the distribution of the F_1/F_0 linearity was significantly different between the sSC and the dSC ($p = 1.8 \times 10^{-6}$, KS test). Figure 8F quantifies the fractions of the C-like nonlinear cells in the sSC and the dSC, further highlighting the smaller

fraction of linear cells and predominance of the C-like nonlinear cells in the dSC.

Y-like nonlinear cells are restricted to the sSC

The Y-like response property, based on a highly nonlinear spatial summation that is well suited for the detection of small

moving objects or finely textured moving patterns, has not been characterized previously in the mouse brain. We characterized the Y-like nonlinearity with contrast-reversing gratings (Fig. 9A; see Materials and Methods) and found that the Y-like nonlinear cells are absent in the dSC. For this analysis, we used 977 neurons. X-like cells show a response with the same temporal frequency as the stimulus temporal frequency (F_1 component) at low spatial frequencies and this response decays as the spatial frequency increases (Fig. 9B,C). Y-like nonlinear cells show a frequency-doubling response (F_2 component) at high spatial frequencies, as demonstrated in Figure 9, D and E. A significant fraction ($8.3 \pm 1.3\%$) of the cells in the sSC have this Y-like nonlinearity, whereas almost none of the cells ($0.2 \pm 0.2\%$) in the dSC have this property (Fig. 9F).

This result is consistent with the result of C-like nonlinearity, which was predominant in the dSC. The C-like nonlinearity is measured simply at the spatial frequency for a drifting grating at which the neuron's evoked firing rate is maximum, whereas Y-like nonlinearity is measured by observing a transition from a linear response to a nonlinear response as the spatial frequency of a contrast reversing grating stimulus increases. Therefore, the Y-like nonlinearity requires a cell to exhibit both a linear response and a nonlinear response and thus is different from C-like nonlinearity. Indeed, we found that only $0.4 \pm 0.3\%$ of the dSC cells had an F_1 component that is significantly stronger ($p < 0.01$) than the corresponding F_2 component at any measured spatial frequency. Even in response to the drifting gratings stimuli, none of the 473 dSC cells exhibited an F_1 component stronger ($p < 0.01$) than the corresponding F_0 component at any spatial frequency. These two results indicate that linear responses are very rarely observed in the dSC.

Both sSC cells and the dSC cells are modulated by locomotion

The response properties of the sensory neurons are known to be modulated by the state of the animal. For example, locomotion modulates the gain of the visual neurons in mouse V1 (Niell and Stryker, 2010). Because the SC is the other major visual area of the mouse, we speculated that the SC cells are also modulated by locomotion, perhaps in a different manner than the V1 cells. Therefore, we determined the effect of locomotion on the activity of the SC cells in response to the drifting gratings stimuli. We characterized the modulation with an additive-multiplicative (AM) model that includes an additive component (baseline shift) plus a multiplicative component (gain modulation), as described in the Materials and Methods section. To have a sufficient number of trials for both stationary and locomotion periods, we only used experiments in which the mouse spent 20–80% of the total time running. To increase the statistical power, the data from

Figure 10. The activity of the SC neurons is modulated by locomotion. **A**, Example neuron showing a baseline shift (additive) modulation. The blue line is the response during the stationary periods; the red line is the response during the locomotion periods. The purple dotted line is the estimated response fit from the AM model that predicts the activity during locomotion periods based on the activity during stationary periods. The spontaneous firing rates are indicated on the right of the plot. This neuron elevates the firing rate by a constant value over a range of spatial frequencies (baseline shift: 1.5 ± 0.2 Hz, $p = 9 \times 10^{-15}$; gain: 0.5 ± 0.9 dB, $p = 0.54$). **B**, Fractions of the cells that had upward, downward, and no baseline shift. We excluded the cells that did not have a good fit by the AM model ($\chi^2 p < 0.01$). There are cells in both the sSC and the dSC that had an upward baseline shift. The fractional distributions of these cells were only marginally different between the sSC and the dSC ($p = 0.009$ for the unblinded data; $p = 0.35$ for the data initially blinded after unblinding, $p = 0.017$ for the combined data). **C**, Example neuron showing a gain (multiplicative) modulation. This neuron changes the firing rate with a constant factor at different spatial frequencies. (baseline shift: 0.02 ± 0.02 Hz, $p = 0.30$; gain: 3.5 ± 0.5 dB, $p = 5 \times 10^{-15}$). **D**, Equivalent of **B** but for gain modulation. The sSC and dSC distributions are marginally different as well ($p = 0.003$ for unblinded data; $p = 0.66$ for the blinded data after unblinding, $p = 0.011$ for the combined data). **E**, Example neuron that shows a non-AM modulation. This neuron lowers its preferred spatial frequency. **F**, Fractions of the cells that had an upward and a downward frequency shift. A larger fraction of the cells changed the preferred spatial frequency downward.

different stimulus directions were combined, but we evaluated the modulation as a function of the spatial frequency. A total of 607 cells were used for this analysis.

Figure 10, A and C, illustrates the examples of cells that exhibit additive and multiplicative modulations, respectively. The additive modulation is characterized by a constant shift across the spatial frequency range, whereas the multiplicative modulation increases the neuron's firing rate by a constant multiplicative factor independent of the spatial frequency. We also found many cells that are modulated in ways that cannot be described by the AM model. We call these non-AM modulations. An example of a non-AM modulation is shown in Figure 10E. The preferred spatial frequency of this neuron is lowered when the mouse is running. Because of the presence of these non-AM modulations, the AM model did not provide reasonable fits for a large fraction of the cells. A total of $38 \pm 2\%$ of the cells had $\chi^2 p$ -values smaller than 0.01, which indicates underfitting.

The most common type of non-AM modulation is the shift in the preferred spatial frequency of the neurons as shown in Figure 10E. To test whether a neuron changed its preferred frequency, we used the following criteria: (1) the spatial frequency that elicits

the maximum firing rate is different during stationary and running states; (2) the firing rate at the peak spatial frequency is significantly ($p < 0.01$) larger than at least two other spatial frequencies both in the stationary and running states; and (3) the firing rates between the stationary and running states are significantly ($p < 0.01$) different at least in one of the two peak spatial frequencies. The Bonferroni corrections are applied when multiple tests are needed. Of the 232 cells for which the AM model did not fit well, 99 cells ($43 \pm 3\%$) satisfied all three criteria, 33 cells showed an upward frequency shift, and 66 cells showed a downward frequency shift.

The effect of the modulation was only marginally different between the sSC and the dSC (Fig. 10*B,D,F*). The dSC had a relatively larger fraction of cells that received upward additive, multiplicative, and spatial frequency modulations.

To summarize, we found that both sSC and dSC cells are affected by locomotion. A total of $62 \pm 2\%$ of the cell responses were well fit by the AM model. Non-AM modulations include a frequency shift modulation exhibited by $43 \pm 3\%$ of the non-AM cells.

Discussion

In this study, we used high-density silicon probe recordings in alert mice to examine the visual response properties of mouse SC cells, with particular attention to the less characterized dSC cells. We found: (1) a novel pattern of visual response; (2) two visual response properties not previously reported in the mouse SC cells; (3) major differences between the visual functional organization of the sSC and the dSC; and (4) three different types of modulation in the visual response properties of SC neurons due to the locomotion of the mouse.

The novel pattern of visual response is the encoding of a cell's preferred orientation/direction in response to drifting sinusoidal gratings stimuli by a negative change of the firing rate. The two, not previously reported, mouse SC response properties are SuC responses and Y-like spatial summation nonlinearity. The sSC is enriched with cells with small RFs, high evoked FRs, and sustained temporal responses with early onsets. In contrast, the dSC is enriched with the negative OS/DS cells and with cells with large RFs, low evoked FRs, and transient temporal responses with late onsets. Almost all of the dSC cells have C-like nonlinearity, but the cells with Y-like nonlinearity are present only in the sSC. Locomotion modulates the activity of the SC cells both additively and multiplicatively and some neurons change their preferred spatial frequency in response to drifting gratings stimuli during locomotion. These results provide important and novel details about the visual coding properties of the mouse SC.

Negative encoding of visual information

Neurons in the early visual system [i.e., the retina, dorsal lateral geniculate nucleus (dLGN), V1, and sSC] typically encode information with a positive change of firing rate. However, there are cases in which nonpreferred stimuli lead to inhibition. In most cases, cells that are inhibited also exhibit excitation to the preferred stimuli. A known exception to this positive encoding are the SuC cells, which reduce their firing rate with a high-contrast stimulus. In mice, SuC cells exist in the retina (Tien et al., 2015), dLGN (Piscopo et al., 2013), and V1 (Niell and Stryker, 2010). Not surprisingly, we found that these cells also exist in the SC, comprising $6.1 \pm 1.4\%$ of the recorded cells in the sSC and increasing to $16 \pm 2\%$ in the dSC.

Independent of the SuC cells, we found that $58 \pm 4\%$ of OS/DS cells in the dSC are “negative” OS/DS cells, in contrast to

the $25 \pm 2\%$ detected in the sSC. The negative OS/DS responses have not been reported previously. These negative responses are elicited by full-field moving gratings stimuli. The majority of the negative OS/DS cells show a positive firing rate change, whereas $14 \pm 3\%$ showed a negative firing rate change, in response to a localized flashing-spot stimulus. This suggests that both the RF center and the surround can contribute to the negative responses of these negative OS/DS cells.

We used localized flashing spots to demonstrate the effect of the surround suppression of the negative OS/DS cells. This approach should work well as long as the system is linear, but the neurons may receive complex nonlinear contributions from outside of the RF, as is observed in V1 cells (Fitzpatrick, 2000). Given that many of the neurons in the SC exhibit nonlinear properties, our linear approach may not give an accurate description of the surround suppression. More appropriate visual stimuli, such as optimally sized localized patches of drifting gratings and/or surround-only drifting gratings, could be used in future experiments to determine in more detail the surround suppression, orientation-selective, direction-selective, and RF properties of the negative OS/DS cells.

We speculate that some of the negative OS/DS cells are narrow field (NF) cells, one of the sSC cell types identified in Gale and Murphy (2014). NF cells receive strong surround suppression, are often DS, and project directly to the dSC. Full-field gratings did not elicit spikes for most of the NF cells, but the investigators did not analyze the negative changes of the firing rate. The properties of the NF cells are similar to the negative OS/DS cells that show surround suppression.

Increased C-like nonlinearity and weaker response to flashing spots indicate that the mouse dSC cells are selective to visual features

The dSC is characterized by the predominance of C-like nonlinearity and weak and transient responses with large RFs to flashing spots compared with the sSC. Interestingly, the transitions from the sSC to the dSC are similar to those between the cortical simple and complex cells (nonlinearity: Skottun et al., 1991; weak, transient response: Hubel and Wiesel, 1962; large RFs: Hubel and Wiesel, 1968). Furthermore, in the mouse, both the SC and V1 are laminar structures with their output layers enriched with complex cells (deep layers of the SC, as reported here, and layer 5 of V1, as reported in Niell and Stryker, 2008). This result suggests that the dSC performs visual feature extraction like that proposed for complex cells in V1.

The differences in visual “selectivity” between the primate SC and the mouse SC also support the hypothesis that the mouse SC plays an extended role compared with the primate SC. The visual response properties of the majority of the deep and superficial monkey SC visually responsive cells have been described as “event detectors”: they respond to almost any stimuli in their RFs (nonselective to visual features) if the stimulus size is appropriate (Schiller and Koerner, 1971; Humphrey, 1968; Cynader and Berman, 1972; Goldberg and Wurtz, 1972). This type of visual response property is useful for calling attention to the appearance of an object and encoding its location for an accurate gaze shift toward the object (Boehnke and Munoz, 2008), but is not useful for finding out what the object is.

Conversely, recent studies of anesthetized mice have shown that the sSC cells are “selective” for visual features (Wang et al., 2010; Gale and Murphy, 2014). We have confirmed visual selectivity in the SC of awake mice and find that the dSC cells are even more selective than the sSC cells. A recent study showed that the

DS retinal ganglion cells (RGCs) are the source of direction selectivity of the mouse SC cells (Shi et al., 2017). We do not know whether other selectivity is also inherited from the retina, but it is worth noting that there are two important differences between the primate and mouse SCs. First, the primate SC is innervated by only ~10% of the RGCs (May, 2006), whereas the mouse SC is innervated by ~88% of the RGCs (Ellis et al., 2016), which includes the DS-RGCs (Gauvain and Murphy, 2015). Second, the mouse does not have a fovea. Nevertheless, the mouse SC cells are more similar to the mouse or primate V1 cells than to the primate SC cells in the sense that they are selective to visual features. This property may support the hypothesis that the SC is important for a number of visually guided behaviors of the mouse (Liang et al., 2015; Shang et al., 2015; Wei et al., 2015), as well as the surprising visual task capability of mice that develop without a visual cortex (Shanks et al., 2016). Our results support the notion, as suggested in Huberman and Niell (2011), that, at least in the mouse, much of the visual functionality that is usually attributed to the cortex is achieved by the SC.

Cells with a Y-like nonlinear spatial summation property are found only in the sSC

We found cells with a Y-like nonlinearity in the sSC ($8.3 \pm 1.3\%$), but not in the dSC ($0.2 \pm 0.2\%$). This Y-like nonlinearity is a well known property of the α /Y-type RGCs, which were originally found in the cat retina (Hochstein and Shapley, 1976) and subsequently in primates (de Monasterio, 1978; Petrusca et al., 2007), guinea pigs (Demb et al., 1999), and mice (Stone and Pinto, 1993). The Y-like nonlinearity of the sSC cells could be inherited from these RGCs. One of the α RGC types in mice, the transient OFF- α RGCs (PV-5 RGCs), project to the sSC (Yi et al., 2012) and show strong sensitivity to a looming spot stimulus, which imitates an approaching object (Münch et al., 2009). This stimulus causes an innate defensive response (Yilmaz and Meister, 2013) proposed to be through a retina-SC-lateral posterior nucleus (LP)-amygdala pathway (Shang et al., 2015; Wei et al., 2015). The SC cells that project to the LP have been identified as the wide-field cells that do not project to the dSC (Gale and Murphy, 2014); this is consistent with our inability to detect dSC cells with these Y-like properties. Therefore, our results are consistent with the hypothesis that the wide-field cells in the sSC obtain their Y-like nonlinearity from the retina and transmit this nonlinearity to the LP, but not to the dSC.

Novel modulation of preferred spatial frequency in the SC during locomotion

We found that locomotion can modulate the activity of many visually responsive neurons in the SC. Most of the SC cells receive additive, multiplicative and/or non-AM modulations due to locomotion. The non-AM modulation includes a shift of the preferred spatial frequency in response to drifting gratings stimuli and, in a larger fraction of the SC cells, this shift in spatial frequency was downward. These modulations are different from those reported in V1, where cells do not change their preferred spatial frequencies, and the cells that prefer a high spatial frequency receive a higher gain modulation (Mineault et al., 2016). This result is surprising because V1 is known to project directly to the SC (Triplett et al., 2009; Wang and Burkhalter, 2013) and suggests that there are other sources of input to the SC that modulate the SC response properties during locomotion. Such differences may be a consequence of the different roles played by V1 and the SC in visual information processing.

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