Laminar Organization of Response Properties in Primary Visual Cortex of the Gray Squirrel (*Sciurus carolinensis*)

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Heimel, J. Alexander, Stephen D. Van Hooser, and Sacha B. Nelson. Laminar organization of response properties in primary visual cortex of the gray squirrel (*Sciurus carolinensis*). *J Neurophysiol* 94: 3538–3554, 2005. First published July 6, 2005; doi:10.1152/jn.00106.2005. The gray squirrel (*Sciurus carolinensis*) is a diurnal highly visual rodent with a cone-rich retina. To determine which features of visual cortex are common to highly visual mammals and which are restricted to non-rodent species, we studied the laminar organization of response properties in primary visual area V1 of isoflurane-anesthetized squirrels using extra-cellular single-unit recording and sinusoidal grating stimuli. Of the responsive cells, 75% were tuned for orientation. Only 10% were directionally selective, almost all in layer 6, a layer receiving direct input from the dorsal lateral geniculate nucleus (LGN). Cone opponency was widespread but almost absent from layer 6. Median optimal spatial frequency tuning was 0.21 cycles/°. Median optimal temporal frequency a high 5.3 Hz. Layer 4 had the highest percentage of simple cells and shortest latency (26 ms). Layers 2/3 had the lowest spontaneous activity and highest temporal frequency tuning. Layer 5 had the broadest spatial frequency tuning and most spontaneous activity. At the layer 4/5 border were sustained cells with high cone opponency. Simple cells, determined by modulation to drifting sinusoidal gratings, responded with shorter latencies, were more selective for orientation and direction, and were tuned to lower spatial frequencies. A comparison with other mammals shows that although the laminar organization of orientation selectivity is variable, the cortical input layers contain more linear cells in most mammals. Nocturnal mammals appear to have more orientation-selective neurons in V1 than diurnal mammals of similar size.

**INTRODUCTION**

A major feature of sensory cortex is the presence of columns of neurons with similar response properties. Most neurons in a vertical column of primary visual cortex (V1) of mammals as diverse as carnivores, primates, and tree shrews respond best to stimuli presented at a narrow range of orientations. These columns are organized horizontally to form smooth, repeating functional maps of orientation preference across the cortical surface. Rodents, however, appear to lack such maps. Recently, we showed that even a large and highly visual rodent, the gray squirrel (*Sciurus carolinensis*), lacks this horizontal organization (Van Hooser et al. 2005). Another major feature of V1 is its laminar division into horizontal layers containing neurons that vary in their functional response properties. This is particularly evident in animals that have been traditionally used for vision research such as the cat and the monkey. In murid rodents, anatomical lamination is less prominent, and there is an absence of pronounced functional laminar organization. For instance, in mouse and rat, direction selectivity is almost uniform across layers (Girman et al. 1999; Metin et al. 1988) unlike cats and primates in which specific sublayers have high direction selectivity (DeBruyn et al. 1993; Gilbert 1977; Hawken et al. 1988; Orban et al. 1986; Snodderly and Gur 1995). Another example is that although many studies find high proportions of simple cells in layer 4 (and 6) of area 17 in the cat (Bullier and Henry 1979; Henry et al. 1979; Hubel and Wiesel 1962; LeVay et al. 1987) and macaque (Hubel and Wiesel 1968; Kagan et al. 2002; Livingstone and Hubel 1984; Ringach et al. 2002; Schiller et al. 1976a), so far only one study has found a similar trend in a rodent species (Parnavelas et al. 1983). These differences in laminar specificity might be due to differences in animal size or to the degree of specialization for vision. On the other hand, it may be the case that all rodents lack a strong laminar organization in V1. If so, this would indicate a more general lack of functional organization in rodent visual cortex and would suggest that mechanisms for vertical and horizontal organization are related or interdependent.

To answer this question, we studied the vertical organization of a highly visual rodent, the gray squirrel. This animal has large eyes and relatively large and well-laminated visual brain structures (Hall et al. 1971; Van Hooser et al. 2003). Its visual acuity [2.8–3.9 cycles/° (cpd)] (Jacobs et al. 1982) is better than a tree shrew’s (1.2–2.4 cpd) (Petry et al. 1984), and its visual cortex is comparable in size to a ferret’s (Hall et al. 1971; Law et al. 1988). Orientation selectivity is very common among V1 neurons of squirrels (Polkoshnikov and Supin 1988; Van Hooser et al. 2005).

Unlike nocturnal rodents, gray squirrels have good color vision. The gray squirrel retina contains rods and cones in a 2:3 ratio (Anderson and Fisher 1976; Cohen 1964; West and Dowling 1975). It contains two classes of cones, a short wavelength S-cone with a peak sensitivity of ~444 nm and a medium wavelength M-cone with a peak sensitivity of ~543 nm and a rod photoreceptor with a peak sensitivity at ~502 nm (Blakeslee et al. 1988). Gray squirrels also have a much higher percentage of color-selective optic nerve fibers than cats have (6 vs. <1%) (Blakeslee et al. 1988; Pearlman and Daw 1970). This provides an extra opportunity for a comparative study of dichromatic color vision outside the primate order.

Apart from laminar variations (see e.g., Table 2), there are also differences in the overall degree of selectivity for different stimulus features. Perhaps the most obvious are the large differences across mammals in the spatial acuity of neurons in...
V1. This most likely reflects differences in eye size and retinal ganglion cell density. Other differences, such as the varying level of orientation selectivity and temporal frequency tuning across species are not easily explained by differences in size (see Table 2 for numbers and references). A pattern may emerge if we would have knowledge of the response properties of a diurnal rodent.

For these reasons, we studied the organization of response properties in V1 of the gray squirrel and focused on their laminar organization. We found several functional cell types that have been reported in other mammals: orientation-selective cells, direction-selective cells, end-stopped cells, and cone-opponent cells. Based on the linearity of the response, we found both simple and complex cells. Simple cells were more common in the major input layers from lateral geniculate nucleus (LGN), while orientation selectivity did not vary across layers. Most cells received input from S and M cones and showed some degree of both luminance and color sensitivity. High direction selectivity and cone opponency were mutually exclusive, and layer 6 contained many direction-selective cells and almost no cone-opponent cells. The distribution of response properties in gray squirrel V1 is broad and lacks clear classes, but there are correlations between response properties and laminar location. Thus the lack of functional horizontal organization is not accompanied by poor laminar organization in all rodent species. We find that a prominent laminar organization is present in all highly visual mammals, suggesting that the mechanism for organization of V1 into smoothly ordered orientation columns differ from the mechanisms that produce laminar functional organization of this area.

METHODS

Surgical preparation and maintenance

Adult gray squirrels (Sciurus carolinensis) weighing 300–700 g were prepared for single-unit recording using methods similar to those of Van Hooser et al. (2003). Animals were initially anesthetized with a mixture of ketamine and acepromazine maleate (90 mg/ml ketamine, 0.91 mg/ml acepromazine maleate, 0.5 ml/kg initial dose im). A tracheotomy was performed for artificial ventilation and administration of isoflurane anesthesia after surgery (0.5–1.5% isoflurane in 50/50 oxygen/nitrous oxide). End tidal CO2 was measured and kept at 4% by adjusting the ventilation rate within 35–90 strokes/min. The animal was mounted in a custom stereotaxic frame. Rectal temperature was recorded and maintained at 38°C. Electroencephalography (EEG) and heart rate were monitored continuously to assess the level of anesthesia. If spindle activity disappeared from the EEG or if the heart rate changed in response to a toe pinch, the isoflurane concentration was increased. If the EEG showed long periods of low activity, the level was decreased. Typically 1% isoflurane was adequate to maintain this level of anesthesia. Preparations were generally stable for >30 h. To minimize eye movements, a paralytic agent (10 mg/ml gallamine triethiodide, 0.5 ml/h iv) was infused. Eyelids were held open with loose sutures. Pupils were dilated with 1% atropine sulfate. Plano contact lenses were inserted to prevent drying. In earlier experiments (Van Hooser et al. 2003), we found that it was not necessary to adjust the focus of the eyes. All procedures were approved by the animal care and use committee at Brandeis University.

Recording

The primary visual cortex in the gray squirrel is a large area surrounding the posterior medial pole of the cortex (Hall et al. 1971). Part of it is folded beneath the brain surface, but all of the central vision representation is easily accessible. The anterior-lateral border of V1 represents the vertical meridian. We made a craniotomy (~3 × 3 mm3) centered on average at 6.5 mm posterior and 3.5 mm lateral from bregma. In early experiments, when recording close the vertical meridian, we mapped the progression of receptive field positions at several locations to ascertain that we were in V1 and not in neighboring V2, which has a mirrored retinotopy. Under our anesthesia regime, we learned that we could easily determine whether we were in V1 or V2 because the amount of multunit response to visual stimuli in and around layer 4 is much lower in V2 (Hall et al. 1971). In the first half of the experiments, the dura was resected, and later the dura was left intact. Warm artificial cerebral spinal fluid was used to keep the brain moist, and 3% agar in lactated Ringer solution was used to protect the brain and minimize pulsations. We used high-impedance micro-electrodes (10 MΩ, FHC, Bowdoinham, ME) and a custom-developed multiple window discriminator to isolate single units. We recorded data on 21 penetrations that were roughly perpendicular to the cortical surface. We searched for well-isolated units, so our sample may be biased toward larger cells. After recording from one cell, the electrode was advanced ±40 μm, and a subsequent cell was included if the electrode had advanced ±100 μm or if the cell showed different response properties from the previous cell. Histological reconstructions showed that penetrations were generally within 15° of perpendicular to the cortical surface (Van Hooser et al. 2005).

Reconstruction of recording sites

At the end of each penetration, three electrolytic lesions (9 μA, 3 s, tip negative) were made along the electrode track to recover the recording positions. The electrodes were coated in DiI (Molecular Probes, Eugene OR) so that the tracks could later be found (DiCarlo et al. 1996; Snodderly and Gurn 1995). The animals were given an overdose of ketamine/acepromazine or sodium thiopental and perfused transcardially with 0.1 M phosphate-buffered solution (PBS) followed by 4% paraformaldehyde. Coronal sections (50 μm) were prepared on a cryostat. Sections were Nissl-stained, as previously described (Van Hooser et al. 2005). Cortical layers 2/3–6 could easily be identified, as shown in Fig. 1. Sublayers

![FIG. 1. Reconstructing an electrode track. Nissl-stained coronal section of primary visual cortex (V1) with DiI-coated microelectrode track and electrolytic lesion. Layers 2/3–6, and white matter are distinguishable.](image)
3c and 6b (Kaas et al. 1972b; Moore 2001) could often be distinguished and occasionally a sub-lamination of layer 4 was apparent but not consistently enough to include this in our laminar analysis. Cytochrome oxidase staining did not reveal any further sublamination or tangential organization (Moore 2001). We used the lesion sites to calibrate the electrode manipulator readings to laminar positions in the cortex using a technique similar to (Hawken et al. 1988). Recording sites were assigned a layer and a relative depth within that layer. By studying lesions and dye labeling from the same penetration and laminar borders on several neighboring sections, we estimate that the error in the reconstruction depth is ~70 µm. For seven penetrations, we could not reconstruct the relative depths for the recording sites with certainty and left these cells out of the laminar analysis.

**Visual stimulation**

Visual stimulation was provided by an Apple Macintosh PowerMac G4 and a gamma-corrected Samsung SyncMaster 900SE CRT monitor running custom-developed stimulation software (Van Hooser et al. 2003) using Matlab (The MathWorks, Natick, MA) and the Psychophysics Toolbox (Brainard 1997; Pelli 1997). Stimuli were shown at a distance of 57 cm from the eyes with a refresh rate of 120 Hz.

**Color stimulation**

We measured the spectra of the red, green, and blue phosphors (denoted R, G, and B) on our monitor from 400 to 700 nm in steps of 4 nm using a PR-650 spectral radiometer (Photo Research, Chatsworth, CA) kindly lent to us by R. Clay Reid of Harvard University. Using activity spectra S and M for the blue and green cones, respectively, estimated from Blakeslee et al. (1988) and correcting these values for preretinal absorbance (Yolton et al. 1974), we computed a matrix relating RGB color values (r, g, b) to relative cone activation (S, M) = (r S, G S, B S, R M, G M, B M) · (r; g; b) = (0.0008, 0.00057, 0.0299; 0.0135, 0.0742, 0.0191) · (r; g; b). Using this equation we computed an "equiluminant" pair of colors, blue (r, g, b) = (0, 0, 1) and green (1, 0, 42, 0). The blue color mainly activates the S cone, whereas the green color mainly activates the M cone. The total activation of the two cone types, (S + M), is very similar for the two colors. The luminance contrast of this stimulus (computed using this total activation) is only 2%.

We also used cone-isolating stimuli (Estevz and Spekreijse 1982) to assay the cone inputs to a subset of the recorded cells. We developed one pair of colors for each cone: m and m for the M cone, and s and s for the S cone. The color pairs were chosen so that a transition from one color to the other would dramatically change the drive to the cone of interest while minimally influencing the drive to the other cone. Thus switching from s to s dramatically increases the drive to S cones, but M cones respond with equal intensity to both s and s. We found color pairs that meet the cone isolating criteria and provide strong contrast: m (1, 1, 0), m (0, 0, 0.24), s (0, 0.98, 0.11), 0). The m and s pairs correspond to 90 and 10% M-cone contrast and 5 and 90% S-cone contrast. The luminance contrast between the pairs (using dF/mean L) is 96% for m and m, and 95% for s and s. The pairs do not provide the maximum possible contrasts because they were originally designed using less accurate measurements of the CRT phosphor spectra.

It is difficult to assess the validity of our computations psychophysically. We have simulated a behavioral experiment by Blakeslee et al. (1988) in which gray squirrels were required to identify a colored light among three choices: two achromatic lights (4,800 K temperature) and a monochromatic light that varied in wavelength from 470 to 510 nm. The investigators observed a clear neutral range between 495 and 505 nm where the gray squirrels could not distinguish the colored light from achromatic light. We computed the ratio of green to blue cone activation for a 4,800 K source using the cone spectra estimated above and correcting for retinal absorbance, and then computed the same ratio for stimulation with monochromatic light. The achromatic and monochromatic activation ratios were equal for a 502-nm monochromatic stimulus, which is in excellent agreement with the neutral range in the preceding text, suggesting our choices for cone-isolating stimuli are appropriate. In addition, in this study and in pilot experiments in the LGN we encountered some cells that responded exclusively to either the S- or M-cone isolating stimuli, consistent with the idea that these stimuli are truly cone isolating.

**Experimental protocol**

Receptive fields were initially mapped by hand on a tangent screen. Stimuli were presented monocularly to the dominant eye. We did not score ocular dominance or binocularity, as receptive fields in the two eyes did not always overlap, possibly due to the paralysis of the animal. Coarse orientation tuning curves were measured using large (~16 × 16°) sinusoidal gratings drifting in 12 equally spaced different directions. These gratings had a spatial frequency of 0.2 cpd and drifted with a temporal frequency of 4 Hz. Most cells responded vigorously to at least one direction. As with all our tests, all stimulus conditions were pseudorandomly interleaved and shown five times. Between all of our stimuli a gray background (luminance: 45 cd/m²) was shown for ≥3 s. Except for the contrast tuning test, contrast was 80% for all tests.

We determined the optimal direction (maximum modulation or firing rate, see following text) and used this for all subsequent stimuli. For two-thirds of our experiments, a small patch of this grating (5 × 5° square rotated to match optimal orientation) was shown at nine overlapping positions around the hand-mapped center to determine the receptive field center more accurately. Centered at this position we showed drifting sinusoidal gratings of nine different spatial frequencies between 0.015 and 1.6 cpd in the original larger window of 16 × 16°. Further tests used gratings with the optimal spatial frequency. Temporal frequency was varied between 0.5 and 32 Hz, and the optimal temporal frequency was used for the remaining tests. Contrast tuning was measured using drifting gratings at seven contrasts levels between 2 and 100%. To determine latency and spatial linearity, we flashed static gratings with 12 different spatial phases.

For a subset of the cells, color sensitivity was assessed by measuring spatial frequency tuning again using an equiluminant colored grating drifting at 4 Hz. For some cells, we measured cone inputs and cone opponency by showing a series of linear combinations of cone-isolating colors, with m, overlapping with s and m with s. In the early spatial phase of the sinusoidal grating, we used the combination m + (1 − m), and in the late phase, we used m + (1 − m). Thus for m = 0.1 the stimulus is S(M)-cone isolating.

The total characterization procedure took a little less than an hour per cell. Not all cells could be kept well isolated long enough to run all tests. The results of all tests run are included in the analysis.

**Data analysis and statistics**

Average firing rates (DC or F₁ component) and response modulation at the drifting frequency (F₀ component) were computed. The parameter value that gave the largest F₁ or F₀ component overall was considered optimal and was used in subsequent tests. For the analysis of the data and fitting of tuning curves, we did not use the F₁ modulation but used the average firing rate for all cells. This allowed us to compare the tuning of cells with different F₁/F₀ ratios.

For several parameters, we defined low and high cut-offs as the parameter value that gave the largest F₁ or F₀ component overall was considered optimal and was used in subsequent tests. For the analysis of the data and fitting of tuning curves, we did not use the F₁ modulation but used the average firing rate for all cells. This allowed us to compare the tuning of cells with different F₁/F₀ ratios.

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often used as a measure of orientation selectivity in V1, see e.g.,
(Worgotter and Eysel 1987). Von Mises functions $\rho_p \left[ 1 + p \exp\left[ \cos(2\theta - \theta_{max}) \right] - 1 \right] / \rho_p$ provide the best fit to orientation tuning curves (Swindale 1998). From Von Mises fits, we computed the tuning width as half width at half the height (HWHH) of the maximum $F_r$ response (Henry et al. 1974). A directionality index (DI) was computed as $(R_{\text{optimal}} - R_{\text{opposite}})/R_{\text{optimal}}$, subtracting the spontaneous firing rate from all responses. Spatial and temporal frequency responses were fit with difference of Gaussians. For the temporal frequency tuning, a constraint was added that $>150$ Hz the cells would respond at the spontaneous rate. The fits were not very sensitive to the point at which this constraint is enforced.

Few, if any, of the response properties were normally distributed. To compare scalar properties between different groups of cells, we used a nonparametric Kruskal-Wallis rank test, unless otherwise stated in the text. To calculate the significance of differences in categorical properties, we applied a $\chi^2$ test. For computation of the significance of bimodality, we used a Matlab implementation of Hartigan’s DIP test (Hartigan and Hartigan 1985) by F. Mechler (available at http://manuelita.psych.ucla.edu/~dario/neurodata.htm). In bar graphs, significance is shown by asterisks, *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

RESULTS

We studied the functional response properties of 221 neurons in the primary visual cortex of 16 gray squirrels using sinusoidal gratings. Of these cells, 27 did not respond to visual stimulation or had peak firing rates $<3$ Hz, and we excluded these from our analysis. Our sample of cells was well distributed across cortical layers. We could reconstruct the locations of 41, 32, 27, and 28 cells in layers 2/3, 4, 5, and 6, respectively. We did not record any cells in layer 1. The same cells were used for one additional test in which the orientation preference and tuning width dependence on contrast was measured. The results of this test and the absence of a smooth horizontal organization of orientation preference were reported earlier (Van Hooser et al. 2005).

Gray squirrel V1 is divided into a lateral binocular zone that mediates the central $30^\circ$ of vision in each hemisphere and a medial monocular zone (Hall et al. 1971; Kaas et al. 1972a). Receptive fields of cells in our study all fell between $-30$ and $10^\circ$ of the horizontal meridian and were $<60^\circ$ lateral of the vertical meridian, and three-quarters (151/194) of these cells were within the binocular zone. Of these, only 8% (12/151) responded preferentially to the ipsilateral eye.

Relative modulation

The relative modulation $F_r/F_{0_r}$ i.e., the ratio of response modulated at the drift frequency to the average firing rate, is a measure of the linearity of input summation of a neuron. Researchers in other mammals have reported a bimodal distribution of $F_r/F_{0_r}$ with a valley near 1 (Schiller et al. 1976c; Skottun et al. 1991). We did not find such a bimodal distribution if all cells are considered (Fig. 2C). A bimodality is apparent if we only consider the more oriented cells, i.e., all cells with an orientation tuning half-width at the half height of the maximum response (HWHH, see Orientation tuning) less than the population median, (Fig. 2C, dark bars), but this was not significant ($P = 0.09$). A bimodality does not necessarily imply the existence of two distinct populations but may reflect a nonlinearity in the relative modulation measure itself that could make a unimodal distribution of cell parameters appear bimodal (Mata and Ringach 2005; Mechler and Ringach 2002). However, because the relative modulation correlates to several other response properties, such as the preferred spatial frequency and the contrast linearity (see following text), it is convenient for presentation purposes, to take the $F_r/F_{0_r} = 1$ as a boundary between two classes. We did not measure subfields and cannot apply the original definition of simple and complex cells based on subfield segregation (Hubel and Wiesel 1962), but the 146 cells with smaller $F_r/F_{0_r}$ values, we call “complex,” and the 48 cells with ratios above this value we call “simple.”

Figure 2, A and B, shows the laminar distribution of the relative modulation. The dots in Fig. 2A show the individual cells. The black line is the median over a window with a width of $12\%$ of the cortical thickness (corresponding to $\sim 250 \mu$m). The gray lines on either side are first and third quartiles calculated over the same moving window. Figure 2B shows the same data grouped per layer. The height of the bars corresponds to the median with the lower and top end of the lines corresponding to the first and third quartiles. This convention is followed in all the graphs in this paper unless otherwise stated. The $F_r/F_{0_r}$ ratio is higher in layer 4 than in the other layers, (median: 0.81 vs. 0.58 average across layers), but the difference is not significant ($P = 0.12$). Layers 4 and 6 together, the two layers receiving densest innervation from the LGN, do have a significantly higher $F_r/F_{0_r}$ ratio ($P = 0.01$).

The response to flashed stationary gratings at different spatial phases is another indication of the spatial linearity of a cell. The spatial linearity is defined as the ratio of the spatial $F_1$ component and spatial $F_0$ component of the response. The measure is very similar to the $F_r/F_{0_r}$ value computed for
drifting gratings, and the two measures are highly correlated ($r = 0.6$, $P < 10^{-11}$). Its distribution is very skewed and only shows a hint of bimodality with a notch around $F_1/F_0 = 0.75$. The laminar distribution, shown in Fig. 2D, is similar to that of the temporal modulation ratio of $B$, but the differences are more pronounced. Layers 2/3 and 5 are on average more nonlinear than layers 4 and 6.

When making the distinction between simple and complex cells based on relative modulation due to moving stimuli in primates, one finds much higher percentages of simple cells than when using static stimuli and subfield segregation for the classification (Bullier and Henry 1980; Kagan et al. 2002). There is no such difference between the two measures within our dataset: a quarter (26%) of the cells are simple based on the criterion that (temporal) $F_1/F_0 > 1$ for drifting gratings, whereas 27% have (spatial) $F_1/F_0 > 0.75$ for static gratings.

**Orientation tuning**

The degree of orientation tuning varied enormously, from cells that were completely indifferent to orientation, e.g., the example shown in Fig. 3A, top, to cells that responded very selectively to certain orientations, e.g., Fig. 3A, bottom. The median, first and third quartiles of the OSI are 0.29, 0.13, and 0.54 as shown in the cumulative histogram in Fig. 3B by the dashed and dotted vertical lines.

The HWHH of the maximum $F_0$ response gives information about selectivity around the peak, whereas the OSI measures the strength of the oriented response relative to responses at all angles. The median HWHH is 32°, see Fig. 3C. About 25% of the cells are not orientation-selective and have HWHH $\approx 90^\circ$. Both the OSI and HWHH show a significant difference in the orientation selectivity of simple and complex cells. The median OSI of simple cells is 0.5, whereas that of complex cells is 0.25 ($P = 0.0002$).

We found little difference in the distribution of orientation selectivity across the different layers of the cortex, as shown in Fig. 3D. An analysis of the horizontal organization across the cortical surface of orientation preferences of cells in this dataset has been published previously (Van Hooser et al. 2005).

**Direction tuning**

The median DI is a low 0.23, Fig. 3E. Using the criterion of DI $> 2/3$, only 10% of the cells are directionally selective. The directional selectivity is significantly higher in layer 6 than in other layers as illustrated in Fig. 3F ($P = 0.0001$). Simple cells are more directional than complex cells (median: 0.34 vs. 0.18, $P = 0.001$). The given numbers and statistics are very similar if the spontaneous rate is not subtracted (not shown).

**Spatial frequency tuning**

Spatial frequency tuning with drifting gratings in the optimal direction was recorded in 192 neurons and fit with a difference of Gaussians. The median optimal spatial frequency is 0.21 cpd with complex cells preferring slightly higher frequencies than simple cells ($P = 0.006$; Fig. 4B, 2 middle curves). This difference is more pronounced for the high cut-off spatial frequency shown by the right two curves in Fig. 4B and Table 1 ($P = 0.0002$). The high- and low-frequency cut-off frequencies (frequencies with responses of half the maximum re-
means that in general, cells do not sacrifice selectivity in one of the preferred spatial frequencies. An illustration of this is that the preferred spatial frequency is even negatively correlated with the orientation tuning width ($r = -0.32, P < 10^{-5}$).

**Temporal frequency tuning**

Using drifting gratings, we measured the cells’ temporal frequency tuning from 0.5–32 Hz. About a third of the cells fired significantly above the spontaneous rate at 32 Hz, but as our monitor had a refresh rate of 120 Hz, we could not test cells at much higher frequencies. A fairly typical example of the temporal tuning of a band-pass neuron is shown in Fig. 5A. Median optimal frequency is 5.3 Hz. Simple and complex cells do not differ from each other in their temporal frequency tuning, see Fig. 5B. Median high temporal frequency cut-off is 17 Hz. At the lowest temporal frequency (0.5 Hz), 10% of the cells still respond above half of the maximum response. These features to obtain higher selectivity in other features.

### Summary of results

<table>
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<th>Modulation</th>
<th>Layer</th>
<th>R.F. Location</th>
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<td>Simple</td>
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<tr>
<td>2/3</td>
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<td>Directional Index (DI)</td>
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<td>0.21**</td>
<td>0.21</td>
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<td>34*</td>
<td>39</td>
</tr>
<tr>
<td>Relative max. gain</td>
<td>2.4</td>
<td>2.8**</td>
<td>2.4</td>
</tr>
<tr>
<td>Color and equiluminant grating</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optimal spatial frequency</td>
<td>0.11</td>
<td>0.17</td>
<td>0.15</td>
</tr>
<tr>
<td>High cut-off</td>
<td>0.30</td>
<td>0.34</td>
<td>0.33</td>
</tr>
<tr>
<td>Percentage lowpass</td>
<td>22</td>
<td>32</td>
<td>15</td>
</tr>
<tr>
<td>Color sensitivity</td>
<td>0.59</td>
<td>0.75*</td>
<td>0.76</td>
</tr>
<tr>
<td>Cone balance $\eta_{\text{min}}$</td>
<td>0.25</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>Percentage Cone-opponent</td>
<td>47</td>
<td>60</td>
<td>52</td>
</tr>
</tbody>
</table>

Values given are medians. Significances are indicated by *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$. 

**Color and equiluminant grating** were estimated using the fit. If a cell’s response did not drop below half the maximum response for the lowest frequency that we used in our tests (0.015 \(\text{cpd}\)), the cell was classified as low-pass. The majority (82\%) of the cells show band-pass spatial frequency tuning like the example shown in Fig. 4A, top. The median bandwidth log$_2$(high cut-off frequency/low cut-off frequency) of the band-pass cells is 2.1 octaves. The full distribution of bandwidths is shown in Fig. 4C. There is very little laminar dependence of the preferred spatial frequency. A broadening of the distribution is seen in layer 5. Layer 5 also contains a higher percentage of low-pass cells than other layers, Fig. 4D, but this was not significant ($P = 0.12$) and there is hardly any variation of the high cut-off spatial frequency.

The highest preferred spatial frequencies are found centrally within the visual field. More peripherally the preferred spatial frequencies drop significantly. For the 144 cells within the central 30° of the visual field, the median preferred spatial frequency is 0.23 \(\text{cpd}\), whereas for the 48 cells in our sample outside that region, the median is 0.17 \(\text{cpd}\) ($P < 10^{-5}$).

The spatial frequency bandwidth is positively correlated with the orientation tuning width ($r = 0.26, P < 10^{-5}$) and the temporal frequency bandwidth ($r = 0.25, P = 0.002$). This means that in general, cells do not sacrifice selectivity in one of these features to obtain higher selectivity in other features.
cut-off (calculated as the high temporal frequency cut-off divided by the optimal spatial frequency) median is 86°/s.

Cells in layers 2/3 have a higher optimal temporal frequency ($P/H110050.004$), Fig. 5D, and a higher low cut-off frequency ($P/H110050.0002$), E, than cells in other layers. There are no significant laminar differences in the high temporal cut-off frequency. Optimal velocity is significantly higher in layers 2/3 (median: 31°/s, $P/H110050.006$) and lower in layer 4 (median: 19°/s, $P/H110050.04$).

The temporal frequency tuning of cells responding to the central 30° of vision (median: 6.2 Hz) and those responding to the periphery (median 5.3 Hz) is not significantly different. Preferred velocities are significantly higher in the periphery than in central vision because cells mediating central vision have higher preferred spatial frequencies than those in the periphery.

Responses to static stimuli

LATENCY. After determining a cell’s optimal parameters for drifting gratings, we used these parameters to show stationary gratings. The spatial phase with the strongest response within 1s was used to calculate the cell’s latency, defined as the time at which the firing rate reached half its maximum value. The latency is strongly laminar dependent, as can be seen in Fig. 6, A and B. The median latency of the whole population is 34 ms, with first and third quartiles of 25 and 52 ms, shown in Fig. 6C. Cells in layer 4 have a significantly shorter latency ($P/H110050.001$) than those in other layers, with a layer median of 26 ms. Layers 2/3 have significantly longer latencies with a median of 48 ms ($P/H110050.0003$).

Simple cells respond much more rapidly than complex cells ($P/H110050.0007$), Fig. 6C. The simple cell median is 25 ms, whereas the complex cell median is 37 ms.

Response duration

Figure 7A shows a few examples of neuronal responses to the onset of stationary gratings. Some neurons have a very transient response consisting of a single peak with a latency between 18 and 80 ms. Many other cells continue to fire afterward at a lower rate but above spontaneous levels, and some of these cells show a pronounced second wider peak at a latency ~100 ms. We did not see an obvious correlation between the state of the animal and response duration as we often recorded sustained cells after encountering transient cells and vice versa.

To quantify the response properties, we defined a transience index $TI = (R_{\text{early}} - R_{\text{late}})/R_{\text{early}}$. The early response $R_{\text{early}}$ is the spike count in the 50-ms interval after the response latency time minus the expected number of spikes in this period based on the spontaneous firing rate. The late response $R_{\text{late}}$ is calculated similarly using spikes between 300 and 400 ms after the response latency. A transience $TI = 1$ corresponds to the complete absence of sustained firing 300 ms after response onset. The cells in the cumulative histogram shown in Fig. 7B as having $TI > 1$ have a late response below spontaneous

![Figure 5](image1.png)

**Fig. 5.** Temporal frequency. A: example temporal frequency tuning curve. Error bars show SE. The curve is a fit with a difference of Gaussians. Dashed vertical lines (from left to right), low cut-off, optimal and high cut-off frequency. B: cumulative histogram of temporal frequency tuning. Black lines, all cells; gray lines, simple cells; left-most curves, low cut-offs; middle curves, optimal frequencies; right-most curves, high cut-off frequencies. C: bandwidth log$_2$(high cut-off frequency/low cut-off); white bars, complex cells; gray bars, simple cells. D: optimal frequency is higher in layers 2/3 ($P/H110050.004$). E: low cut-off frequency is also higher in layers 2/3 ($P/H110050.0002$)

![Figure 6](image2.png)

**Fig. 6.** Latency. A: depth profile of latency shows strong laminar dependence. B: median and quartiles of latency across layers. Layer 4 latency is shorter and layers 2/3 latency is longer than the average population. C: cumulative histogram. Simple cells (gray curve) tend to have shorter latencies than complex cells (black curve).

![Figure 7](image3.png)

**Fig. 7.** Response to stationary stimuli. A: examples of peri-stimulus histograms of responses after onset of stationary grating at time 0. Numbers give the transience index (see text). B: cumulative histogram of transience values. No difference exists between transience of complex and simple cells. C: transience vs. cortical depth. Layer 4/5 border contains very sustained cells.
levels. The median transience index is 0.9. There is no difference \((P = 0.77)\) between simple and complex cells. Cells in layers 2/3 on average show more transient responses (median: 0.96, \(P = 0.02\)), whereas cells in layer 5 are more sustained (median: 0.74, \(P = 0.01\)). Figure 7C shows that the layer 4/5 border contains many very sustained cells.

**Spontaneous and maximum rates**

Spontaneous firing rates are between 0.01 and 20 Hz, with an overall median rate of 0.46 Hz (see Fig. 8A). Complex cells have significantly higher firing rates than simple cells with a median 0.57 versus 0.22 Hz \((P = 0.006)\). There is a clear laminar dependence. Cells in layer 5 have significantly higher spontaneous rates than the general population, (median: 1.7 Hz, \(P < 10^{-5}\)), whereas layers 2/3 have a much lower firing rate (median: 0.19 Hz, \(P < 10^{-5}\)). The median maximum average firing rate in response to five cycles of an optimal drifting grating of 100% contrast is 19 Hz. There are no significant laminar differences in the maximum average firing rate. Spontaneous and maximum rates are correlated \((r = 0.4, P < 10^{-6})\).

**Contrast tuning**

The three examples in Fig. 9A illustrate the wide range of contrast response curves observed. All curves could be well fit by the nonlinear Naka-Rushton equation (Albrecht and Hamilton 1982; Naka and Rushton 1966)

\[ R = R_s + gC^n(x^b + C_s) \]

where \(R_s\) is the spontaneous rate and \(C\) is the contrast. The fitting parameters are: the contrast at mid-saturation \(s\), the gain \(g\), and the exponent \(n\). For most cells in the LGN a good fit can be achieved with \(n = 1\) (see e.g., Van Hooser et al. 2003), but many cells in our V1 sample are more nonlinear and require fits with higher exponents. From the fits, we could calculate the relative maximum gain (RMG). This is the maximum slope of the graphs in Fig. 9A if the maximum rate minus the spontaneous rate is normalized to 1. The RMG is a measure for the nonlinearity in response to contrast and is much less dependent on the fitting procedure than the exponent \(n\) or the gain \(g\). A completely linear contrast response curve would correspond with \(\text{RMG} = 0.01\%^{-1} = 1\). Any nonlinear curve will have a higher RMG. The median RMG is 2.8. We did not find any simple cells with a very nonlinear contrast response curve as can be seen from the simple cell RMG distribution in Fig. 9B. The general difference between the linearity of complex and simple cells is also clear in their population medians of 2.8 and 2.4 \((P = 0.004)\).

Another measure of response linearity is the contrast value at half the peak response \((C_{50})\), measured directly from the stimulus responses by linear interpolation. The \(C_{50}\) is correlated to the RMG \((r = -0.5, P < 10^{-10})\), but the measures provide different information. The median \(C_{50}\) is 35%, but its range is wide, Fig. 9C. There is a small difference \((P = 0.02)\) between the complex (median: 34%) and simple cell populations (median: 41%). There is a negative correlation \((r = -0.32, P = 10^{-7})\) between the \(C_{50}\) and the maximum rate and a positive correlation between the RMG and the maximum rate \((r = 0.26, P = 0.0004)\). The distributions of both RMG or \(C_{50}\) are almost uniform across cortical layers.

**Color**

We ran two tests specifically designed to study color sensitivity. We calculated an S-cone isolating color pair \(s_+\) and \(s_-\) and an M-cone isolating pair \(m_+\) and \(m_-\). The color \(s_+\) excites the S cone much more than \(s_-\) does, whereas both equally activate the M cone. The pair \(m_+\) and \(m_-\) differentially activate the M cone. At the optimal spatial frequency, we showed a family of drifting grating stimuli that linearly interpolate between the S-cone isolating grating and the M-cone isolating grating (Chatterjee and Callaway 2002; Diller et al. 2004; Johnson et al. 2004). The color of the up-phase was \(\eta m_+ + (1 - \eta)s_-\) and the down-phase was \(\eta m_- + (1 - \eta)s_+\). Thus for \(\eta = 0\), this constitutes an S-cone isolating stimulus; for \(\eta = 1\), an M-cone isolating stimulus at the opposite phase; and intervening values of \(\eta\) are combinations of the two. We also measured the spatial frequency tuning using a pair of equiluminant colors that gave maximally different responses in each cone type while the sum of the two cone-type activity was identical.
To improve our intuition for how spatial profiles of cone input influence the responses to these stimuli, we constructed linear model cells, shown in Fig. 10A, and computed their responses. The graphs in the left column show spatial profiles $W_S(x)$ of S- (solid line) and $W_M(x)$ of M-cone (dashed line) input along the direction of movement of the stimulating grating. The middle graphs show model spatial frequency tuning for luminance contrast gratings in black and the spatial frequency tuning for equiluminant color gratings in gray. The right column shows responses for the mixtures of cone isolating stimuli. These pictures illustrate a few properties of the stimuli. If the profiles of S- and M-cone input are identical up to a constant positive factor, i.e., $[W_S(x)/[W_S(x) + W_M(x)] = c$ everywhere and $0 \leq c \leq 1$, there will be a mixture $\eta_{min}$ of the two cone-isolating stimuli that gives no response, such as in the top figure. For an ideal pair of cone-isolating stimuli that equally modulate the S and M cones, this point would be equal to the relative S-cone weight, i.e., $\eta_{min} = c$, for a linear neuron. If a neuron receives only M-cone input, $\eta_{min} = 0$, whereas only S-cone input corresponds to $\eta_{min} = 1$. When S- and M-cone inputs are almost identical, i.e., $c \approx 1/2$, the response to an equiluminant grating vanishes. In general, if the S- and M-cone inputs are not proportional to each other, a cell responds for all $\eta$. One exception to this rule is when both S- and M-cone inputs are on-like or both are off-like. Even if the spatial profiles are not proportional to each other, there will be a mixture where there is no response, see Fig. 10A, second row. In such a case, the spatial frequency tuning will be low-pass for both black-and-white and colored stimuli. In recognition of these properties, we name nonopponent those cells that have a point in the color mixture stimulus where they do not fire above spontaneous rate (by $\geq 3$ SD) and thus lack cone opponency. Cells that respond above spontaneous rate for mixtures of all $\eta$, we name cone-opponent. Cells are called band-pass if they are spatial band-pass filters for both the contrast luminance and the equiluminant gratings and low-pass otherwise.

Double-opponent cells (Livingstone and Hubel 1984), like the example shown at the bottom of Fig. 10A, would be in the band-pass cone-opponent group. In general, for cells in this class, the response to the luminance contrast grating can still be higher than the response to the equiluminant grating. In Fig. 10B, from top to bottom, a nonopponent band-pass, a nonopponent low-pass, a cone-opponent low-pass, and a cone-opponent band-pass (double-opponent) example are shown. The 120 cells for which we ran both these tests are fairly equally distributed among these classes (see Fig. 10E).

A histogram of the cone balance $\eta_{min}$ is plotted in Fig. 10C. The colors correspond to the colors in E. Most of the cells have a low $\eta_{min}$, which means they respond preferentially to M-cone input, like the top three examples shown in Fig. 10B. The distribution of $\eta_{min}$ is not significantly different for cone-opponent versus nonopponent cells (comparing the dark bars with the lighter bars). The distribution of $\eta_{min}$ is wide but skewed toward low values. The average value of $\eta_{min}$ is 0.21 for cone-opponent cells and 0.22 for nonopponent cells for which it is an approximation to the relative S-cone weight. The majority of cells (68%) show a significant response for all $\eta$, even at $\eta_{min}$, and are classified as cone-opponent. A fifth (26/122) of the cells do not fire ($\geq 3$ SDs) above spontaneous rate for the S-cone isolating stimulus, whereas a 10th (12/122) of the neurons do not respond to the M-cone isolating grating.

Figure 10D shows that the preferred spatial frequency is significantly lower for equiluminant gratings than for luminance contrast gratings (0.14 vs. 0.21 cpd, $P < 10^{-8}$). The percentage of low-pass cells is 29% when measured with equiluminant gratings, whereas it is only 19% measured with a luminance contrast gratings. This suggests that the
spatial antagonism between excitation and suppression is not as well matched per cone type as it is for the summed cone inputs.

The laminar distribution of cell classes, shown in Fig. 10E, reveals a striking absence of cone-opponent cells in layer 6 (significantly different from other layers, \( P = 0.0002 \)). The layer 4/5 border has relatively many cone-opponent cells (not shown).

Cone opponency and orientation tuning are not correlated. Figure 10F shows that the percentage of oriented cells (HWHH < 90°) in the two cone-opponent categories is similar to that in the nonopponent categories (\( P = 0.3 \)). The plot, however, shows that nearly all of the spatial band-pass cells are tuned for orientation while only around half of the low-pass cells are (\( P < 0.0001 \)).

The situation is very different for cone opponency and direction selectivity. Cone-opponent cells have a much lower direction selectivity than nonopponent cells (median: 0.16 vs. 0.38, \( P < 10^{-4} \)). Figure 10G shows that virtually none of the opponent cells have a direction bias (DI > 0.5), which is very different from the nonopponent cells (\( P < 10^{-4} \)). There is no difference in direction selectivity of band- and low-pass cells (\( P = 0.4 \)).

Cone-opponent cells have a higher high cut-off temporal frequency (median: 21 vs. 15 Hz, \( P = 0.01 \)) and a much shorter latency (median: 31 vs. 48 ms, \( P = 0.0001 \)). Only 16% of the nonopponent cells have latencies <34 ms, the overall population median. The cone-opponent cells are also much more sustained (median: 0.81 vs. 0.99, \( P < 0.001 \)) and have a lower \( C_{50} \) (median: 31 vs. 44%, \( P = 10^{-5} \)). The groups of cone-opponent and nonopponent cells were not significantly different in other properties such as \( F_{c}/F_{o} \) ratio, optimal spatial and optimal temporal frequencies.

To compare with Johnson et al. (2001) and Shapley and Hawken (2002), we computed the color sensitivity, defined as the ratio of the maximum firing rate for the preferred equiluminant grating over the maximum rate for the luminance contrast stimulus. The median color sensitivity is 0.71. Three-quarters of the cells (116/154) are in the color-luminance category of Johnson et al. (2001) with color sensitivities between 0.5 and 2. Only one cell in our sample responds to the equiluminant grating at more than twice the rate of the luminance contrast grating. Of course, we may have missed cells that do not respond at all to luminance contrast gratings because we used black-and-white gratings when searching for cells. Color sensitivity is not different in the different cell classes nor is it significantly correlated to direction selectivity or orientation tuning, optimal spatial frequency tuning, temporal frequency tuning, or latency.

Apart from the laminar differences, we did not find a clustering of cone opponency or color sensitivity for nearby neurons on a single penetration. The cone opponency was not significantly different on 9 of 10 penetrations for which we studied cone opponency. The 10th penetration had a lower color opponency but a Kruskal-Wallis \( P \) value of 0.04 only. Color sensitivity varied even less between penetrations. Thus we did not find evidence for dramatic clustering of color processing, although we cannot exclude the possibility of a more subtle organization.

**Multivariate analysis**

Except for the \( F_{c}/F_{o} \) ratio, none of the cells’ individual properties show nontrivial bimodality or clustering seen by eye or by the Hartigan’s dip test (Hartigan and Hartigan 1985). To see if our data suggest the existence of discrete groups of cell types, we looked at the clustering of cells in multi-dimensional space. We took various subsets of the measured properties and projected these to their principal component axes. The Hartigan’s dip test did not report a significant bimodality along any of these projections. This certainly does not exclude the existence of cell classes, but we have not found an indication that there are. There is not one axis that explains most of the variance, suggesting that most properties vary mostly independently. Principal component analysis reflected the correlations that we have already mentioned, such as that between sharper orientation tuning and higher \( F_{c}/F_{o} \) ratio but did not unveil any additional underlying structure.

**Discussion**

We found all the major classes of cell types reported in the V1 of other mammals. Unoriented cells, simple and complex oriented cells, both with and without end-stopping are present in gray squirrel V1. We first compare the individual response properties in the gray squirrel to those in other species before discussing their laminar organization.

**Orientation tuning**

Orientation-selective neurons in the gray squirrel have tuning widths that are very similar to those observed in other mammals. If we consider only the oriented cells (with HWHH < 90°), the median tuning width (HWHH) of gray squirrel V1 neurons is 25°. This is remarkably similar to both carnivores and primates, 19–25° in cat (*Felis domesticus*) (Gilbert 1977; Henry et al. 1974), 21° (mean) in ferret (*Mustela putorius furo*) (Alitto and Usrey 2004), 29° in mink (*Mustela vison*) (LeVay et al. 1987), 27° in baboon (*Papio ursinus*) (Kennedy et al. 1985), 27° in owl monkey (*Aotus trivirgatus*) (O’Keefe et al. 1998) and a median width of 24° at 71% of the maximum response in macaque (*Macaca fascicularis* and *M. mulatta*) (Gur et al. 2004; Ringach et al. 2002).

A quarter of the cells are unoriented and have HWHH of ≈90°. This is much more than in the cat, where unoriented cells are very rare (Baumgartner et al. 1965; Gilbert 1977; Hubel and Wiesel 1962; 1%, Murphy and Berman 1979; 5%, Kato et al. 1978), and a little more than most reports in primate, such as macaque (16% *M. mulatta*, Schiller et al. 1976a; 14% *M. fascicularis*, De Valois et al. 1982b) and owl monkey (10%, O’Keefe et al. 1998), although one study in macaque reports a similar number (31% *M. nemestrina*) (Bullier and Henry 1980). It is similar to the slightly smaller tree shrew (*Tupaia glis*), where also a quarter of the cells is not oriented ( Humphrey and Norton 1980). In comparison with other rodents, the gray squirrel number falls in the middle. The mouse (*Mus musculus*) has many more unoriented neurons in V1, 55% (Drager 1975), 46% (Metin et al. 1988), 49% (Mangini and Pearlman 1980). So has the golden hamster (*Mesocricetus auratus*), 60% (Tiao and Blakemore 1976). In the related red squirrel (*S. vulgaris*), 44% (Polkoshnikov and Supin 1988) of cells sampled in V1 and 17% of cells sampled in the binocular
region (Polkoshnikov and Revischchin 1998) were classified as nonoriented. Both tree squirrel species have more unoriented cells than the latest estimates in the rat (Rattus norvegicus), 16% (Parnavelas et al. 1981) and <7% (Girman et al. 1999). Older studies in the rat differ and find as many as 70% unoriented cells (Shaw et al. 1975; Wiesenfeld and Kornel 1975). Girman et al. (1999) suggest that their use of a different anesthetic, paralyzing the animal, and a smaller craniotomy make their higher estimate of orientation selectivity in the rat more reliable. Table 2 shows orientation tuning across several mammalian species.

The fraction of oriented cells across mammals shows two trends. Our interpretation of the literature is that nocturnal and crepuscular species, which have rod-dominated retina’s or like the owl monkey only have a single cone-type (Jacobs et al. 1993), have more oriented cells than diurnal species of similar size. It is difficult to answer the question whether diurnal animals trade orientation-selective neurons or direction-selective neurons for color-selective neurons. We do see, however, that cone-opponent neurons are just as likely to be strongly orientation-selective as nonopponent neurons. There is also no significant negative correlation between the orientation index and color sensitivity. Another trend is that the smallest animals, mouse (adult weight: 25 g) and hamster (adult weight: 90–150 g), have the most unoriented cells and the largest animals, cat and primate have the fewest. An exception to this is the rat (adult weight: 350 g), which is smaller than gray squirrel (adult weight: 600 g) and has more oriented cells, but the rat is nocturnal and may have more oriented cells than a similarly sized diurnal animal.

### TABLE 2. Rules and selected properties in eight different species

<table>
<thead>
<tr>
<th>Animal Order</th>
<th>Animal Size</th>
<th>Orientation Selectivity</th>
<th>Direction Selectivity</th>
<th>Spatial Frequency Tuning</th>
<th>Simple/Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rodent</td>
<td>Mouse</td>
<td>45–54%</td>
<td>Yes</td>
<td>30%</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>Rodent</td>
<td>84–93%</td>
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<td>25%</td>
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<tr>
<td>Gray squirrel</td>
<td>Rodent</td>
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<td>Yes</td>
<td>21%</td>
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</tr>
<tr>
<td>Ferret</td>
<td>Carnivore</td>
<td>95–99%</td>
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<td>20%</td>
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</tr>
<tr>
<td>Cat</td>
<td>Primate</td>
<td>94%</td>
<td>Yes</td>
<td>24%</td>
<td></td>
</tr>
<tr>
<td>Bush Baby</td>
<td>Primate</td>
<td>90%</td>
<td>Yes</td>
<td>27%</td>
<td></td>
</tr>
<tr>
<td>Owl</td>
<td>Primate</td>
<td>69–95%</td>
<td>Yes</td>
<td>&gt;24%</td>
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<tr>
<td>Macaque</td>
<td>Primate</td>
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<td>Life rhythm</td>
<td></td>
<td></td>
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<td>Nocturnal</td>
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<td></td>
</tr>
<tr>
<td>Diurnal</td>
<td>Crepuscular</td>
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<td></td>
</tr>
<tr>
<td>Orientation selective cells</td>
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<td>Yes</td>
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<td>Median HWWH for oriented cells</td>
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<td>Yes</td>
<td></td>
</tr>
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<td>Layers with lower orientation selectivity</td>
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<td>5/6</td>
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<td>Yes</td>
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<td>Layers with higher orientation selectivity</td>
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<td>2/3</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Direction selective cells (DI &gt; 0.7)</td>
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<td>25%</td>
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<td>Yes</td>
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<td>Layers with higher directional selectivity</td>
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<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Simple/complex</td>
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<tr>
<td>Simple cells have narrower orientation tuning</td>
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<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Simple cells have lower spontaneous rates</td>
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<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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</tr>
<tr>
<td>Simple cells have smaller receptive fields</td>
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<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Simple cells are more direction selective</td>
<td></td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
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<td>Simple cells are tuned to lower spatial frequencies</td>
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<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>Simple cells have higher CSO or lower contrast gains</td>
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<td>Yes</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Spatial frequency tuning</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Optimal spatial frequency</td>
<td></td>
<td>0.1 cpd</td>
<td>0.21 cpd</td>
<td>0.25 cpd</td>
<td></td>
</tr>
<tr>
<td>Spatial frequency bandwidth</td>
<td></td>
<td>2 oc</td>
<td>2.3 oc</td>
<td>1.74 oc</td>
<td></td>
</tr>
<tr>
<td>Layers with higher optimal spatial frequencies</td>
<td></td>
<td>2</td>
<td>5 (NS)</td>
<td>1/2</td>
<td></td>
</tr>
<tr>
<td>Layers with smaller SF bandwidth</td>
<td></td>
<td>2/3</td>
<td>5 None</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Layers with broader SF bandwidth</td>
<td></td>
<td>5 None</td>
<td>2/3-6/7 3C</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Temporal frequency tuning</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean optimal temporal frequency</td>
<td></td>
<td>4 Hz</td>
<td>6.6 Hz</td>
<td>2.4 Hz</td>
<td></td>
</tr>
<tr>
<td>Mean high cut-off temporal frequency</td>
<td></td>
<td>2/3</td>
<td>4 (NS)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Layers with lower optimal TF</td>
<td></td>
<td>2/3</td>
<td>4 (NS)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Layers with higher TF resolution</td>
<td></td>
<td>4</td>
<td>4</td>
<td>2/3</td>
<td></td>
</tr>
</tbody>
</table>

The last column indicates if a rule is common to rodents, carnivores, and primates or if a property is size or life rhythm dependent. Italic bold faced entries are exceptions to these rules. A hyphen indicates that we could not find a report measuring the specific property. A question mark follows a disputed fact, and NS follows non significant differences, but in many instances the significance of a result is not given in the original report. References and species: mouse, Mus musculus (Drager 1975; Mangini and Pearlman 1980; Metin et al. 1988; Prusky and Douglas 2004); rat, Rattus norvegicus (Girman et al. 1999; Keller et al. 2000; Parnavelas et al. 1981, 1983); gray squirrel, Sciurus carolinensis, acuity, Jacobs et al. 1982); ferret, Mustela putorius furo (Alitto and Usrey 2004; Baker et al. 1998; Chapman and Stryker 1993; Usrey et al. 2003); cat, Felis domesticus (Blake et al. 1974; Berman et al. 1987; Bierer and Freeman 2003; Bullier and Henry 1979; Gilbert 1977; Gizzè et al. 1990; Henry et al. 1979; Hubel and Wiesel 1962; Ikeda and Wrights 1975; Jacob et al. 2003; Kato et al. 1978; Leventhal and Hirsch 1978; LeVay et al. 1987; Maffei and Fiorentini 1973; Movshon et al. 1978b; Murphy and Berman 1979; Orban et al. 1981; Pribram et al. 1981; Saul and Hubel 1992; Tolhurst and Thompson 1981); bush baby, Galago crassicaudatus (DeBruyn et al. 1993; Langston et al. 1980); owl monkey, Aotus trivirgatus (Jacobs 1977; O’Keefe et al. 1998); macaque, Macaca mulatta and M. fascicularis (De Valois et al. 1974, 1982a; Foster et al. 1985; Hawken et al. 1988, 1996; Hubel and Wiesel 1968; Kagan et al. 2002; Levitt and Lund 2002; Orban et al. 1986; Ringach et al. 2002; Schiller et al. 1976b, Snodderly and Gurr 1995), M. nemestrina (Bullier and Henry 1980) gave the lowest percentage of oriented cells in the macaque column.
tion and tend to have a low $F_1/F_0$. Many complex cells in the macaque, however, have a high relative modulation (Kagan et al. 2002), and a study in the cat showed that many simple cells have a low modulation ratio (Movshon et al. 1978a). We called the three-quarters of the cells in our sample that had $F_1/F_0 < 1$ “complex” and the remaining quarter “simple.” Our data do not allow us to tell whether simple and complex cells are truly separate cell classes or different ends of a continuous population as suggested recently (Chance et al. 1999; Mata and Ringach 2005; Mechler and Ringach 2002). The simple/complex distinction based on $F_1/F_0$ ratio certainly correlates with several other response properties. The population of simple-like cells with $F_1/F_0 > 1$ responds faster, is more sharply tuned for orientation, has higher direction selectivity, lower spontaneous rate, and lower maximum contrast gain, and responds to lower spatial frequencies. The increased modulation as well as selectivity for orientation and direction, and the lower spontaneous rate of simple cells, could be the effect of a higher spike threshold in these cells compared with complex cells (Priebe et al. 2004).

In gray squirrel, simple cells are more orientation-selective than complex cells. This is also true in cat (Gizzi et al. 1990; Murphy and Berman 1979), ferret (Altitt and Usrey 2004), tree shrew (Kaufmann and Somjen 1979), and macaque (Gur et al. 2004; Schiller et al. 1976b; Ringach et al. 2002). An exception is the wallaby marsupial (Ibbotson and Mark 2003) in which a larger fraction of the population of complex cells was sharply tuned than the simple cell population. In rodents, the situation is unclear, as relatively few of the oriented cells are complex in mouse (Mangini and Pearlman 1980) and rat (Girman et al. 1999). The lower spontaneous rate of simple cells is a universal finding, reported in mouse (Drager 1975), rat (Parnavelas et al. 1981), cat (Pribram et al. 1981), macaque (Schiller et al. 1976a), and wallaby (Ibbotson and Mark 2003).

The question of whether simple and complex cells prefer different spatial frequencies has been studied extensively in cat. Maffei and Fiorentini (1973) reported that complex cells prefer lower spatial frequencies. Other authors do not find any significant difference in preferred spatial frequencies (Ikeda and Wright 1975; Movshon et al. 1978b) or the opposite (Tolhurst and Thompson 1981). Studies in the macaque also find no difference (Foster et al. 1985; Kagan et al. 2002). The owl monkey and bush baby (Galago crassicaudatus) are more like gray squirrel in this respect in that simple cells are tuned to lower spatial frequencies than complex cells (DeBruyn et al. 1993; O’Keefe et al. 1998).

Complex cells are reported to have larger receptive fields in mouse (Drager 1975), cat (Leventhal and Hirsch 1978), and macaque (Hubel and Wiesel 1968; Schiller et al. 1976a), but this was not apparent in our dataset or in the hamster (Tiao and Blakemore 1976).

There was no difference in the optimal velocity of simple and complex cells, but simple cells have a slightly higher cut-off velocity on average. In cat, complex cells respond to higher velocities than simple cells (Movshon 1975; Pettigrew et al. 1968). This discrepancy might be due to our indirect measurement of velocity tuning by varying the temporal frequency of a drifting grating of the optimal spatial frequency rather than true species differences.

Direction selectivity

Compared with other mammals, few cells show strong direction selectivity in gray squirrel. We found less than 1/10 of the cells firing more than three times as much to the preferred direction than to the opposite direction (DI > 2/3). When classified in this way, a third of cells in rat (Girman et al. 1999), macaque (Schiller et al. 1976a), and owl monkey (O’Keefe et al. 1998), a quarter in mouse (Metin et al. 1988) and bush baby (DeBruyn et al. 1993), and a fifth in the rabbit (Chow et al. 1971) are direction-selective (see Table 2). The difference between gray squirrel and other rodents also exhibits itself in the LGN. In rat, some cells are strongly direction-selective (Montero and Brugge 1969), whereas we found hardly any such cells in the gray squirrel LGN (Van Hooser et al. 2003). There are, however, many more direction-selective units in the optic nerve in the gray squirrel (10–19%) (Blakeslee et al. 1985; Cooper and Robson 1966) than there are in rat (1.0%) (Hughes 1980) or cat (1.1%) (Cleland et al. 1973), but all of these fibers project to the superior colliculus and not to the LGN (Michael 1970). Both the gray squirrel and the tree shrew, which is another animal with relatively little V1 direction selectivity (Kaufmann and Somjen 1979), have a relatively large superior colliculus. On the basis of lesion studies, it has been argued that this structure plays a larger role in vision in these animals than it does in primates or carnivores (Diamond 1976). The superior colliculus projects via the pulvinar to extrastriate cortex. The California ground squirrel (Spermophilus beecheyi) has two extrastriate areas (ML and L) that are strongly direction-selective (Paolini and Sereno 1998). These areas, possibly homologous to monkey MT, may exist in gray squirrel and may perform much of the cortical processing of motion information that is done in V1 in some other mammals.

Spatial frequency tuning

The optimal spatial frequency tuning of neurons in gray squirrel V1 (median: 0.21 cpd) is only slightly smaller than that in ferret area 17 (Baker et al. 1998) and is within the range expected from other animals with higher or lower visual acuity, see Table 2. In the rat, which has a behavioral visual acuity between two and three times lower than gray squirrel, the average optimal spatial frequency is 0.1 cpd (Girman et al. 1999). The average optimal spatial frequencies in areas 17 and 18 of the cat, which has a visual acuity about twice that of a gray squirrel, are 0.9 and 0.2 cpd, respectively (Movshon et al. 1978b). Table 2 shows that spatial bandwidths are surprisingly similar for all mammals. Across a 50-fold difference in visual acuity, mean neuronal spatial bandwidths range only from 1.5 octave in the cat and monkey (Foster et al. 1985; Movshon et al. 1978b) to 2.6 octave in the bush baby (DeBruyn et al. 1993), with the high-acuity owl monkey and low-acuity rat in the middle of the range (Girman et al. 1999; O’Keefe et al. 1998). The gray squirrel’s bandwidth of 2.3 octave lies on the upper half of the range. Spatial filtering mechanisms are apparently such that both low and high spatial frequency cut-offs scale with the acuity of the animal. Cells in gray squirrel V1 are much more band-pass than cells in LGN, where there is much less filtering at low spatial frequencies (Van Hooser et al. 2003). This suggests that the high-pass filtering mechanism responsible for the bandwidth in V1 is operating at the cortical
level (Bauman and Bonds 1991; Bredfeldt and Ringach 2002), whereas the high spatial frequency cut-off could be due to limits earlier in the visual system. A simple model where two rectangular subfields, each of a size corresponding to the visual resolution, provide excitation and push-pull inhibition gives a bandwidth of 2 octave, close to the value observed in many species.

Spatial frequency bandwidth in gray squirrel is positively correlated to the temporal frequency bandwidth and the orientation tuning width. Thus more selective cells are generally more selective for all stimulus features. Finally, preferred spatial frequency and orientation tuning width are negatively correlated ($r = -0.3, P < 10^{-5}$). All of the eight cells preferring spatial frequencies $>0.5$ cpd have HWHH $<35^\circ$ (mean: $18^\circ$).

Temporal frequency tuning

Neurons in gray squirrel V1 are tuned to high temporal frequencies. From the animals in Table 2, only the macaque has a higher preferred temporal frequency and a higher cut-off frequency. This high temporal resolution most likely reflects gray squirrel and macaque’s cone-based vision. Cones have much shorter integration times and faster transmission than rods. The long integration and summation times of rods are more suited to low light levels and a nocturnal lifestyle. Even within a species, changing the luminance from the cone-dominated range to the rod-dominated range lowers the temporal resolution (Peterson et al. 2001).

It could be hypothesized that animals such as macaques, gray squirrels, and tree shrews need fast visual processing for an arboreal lifestyle. However, cone dominance seems more related to life in the sun than to life in the trees, as in retina of the ground dwelling, diurnal, California ground squirrel cone to rod ratio is 16:1 (Jacobs et al. 1976), whereas in the foveas of the arboreal, but nocturnal, owl monkey and bush baby, rods outnumber cones by 14 and 40 to 1, respectively (Wikler and Rakic 1990).

Color

We studied color vision using cone-isolating stimuli. These stimuli were based on the assumption that the rod photoreceptors were saturated under our experimental light levels (mean luminance = 45 cd/m$^2$). One physiological experiment using gray squirrels (Blakeslee et al. 1988) did find a rod contribution to sensitivity curves of achromatic optic nerve fibers using a much lower 3 cd/m$^2$ illumination, but in support of our assumption, a behavioral experiment using fox squirrels (Sciurus niger, another tree squirrel) has shown a large drop in wave-length sensitivity and a shift away from the rod spectrum between dark-adapted conditions and background luminance of 2.6 cd/m$^2$ or 7 cd/m$^2$ (Jacobs 1975). Also physiological studies of wavelength sensitivity in achromatic optic nerve fibers in the California ground squirrel have revealed a similar drop and shift between conditions of dark adaptation and background luminance of 3 cd/m$^2$ (Jacobs and Tootell 1981).

Two of three cells (68%) responded to all mixtures of two phase-shifted S- and M-cone isolating gratings. If the rod-input indeed is negligible, the absence of a null-response can only be caused by a difference in the spatial or temporal profiles of the input of the two cone types. These cone-opponent cells will show different response properties when measured with light of different wavelengths, whereas nonopponent cells will show no differences other than those seen by varying the contrast or luminance level.

The cone-opponent cells are not exclusively coding color as they are not less selective for orientation or spatial frequency than the nonopponent population. More than 80% of the band-pass cone-opponent cells, which would include any double-opponent cell, are oriented. Unoriented double-opponent cells, reported in the macaque (Livingstone and Hubel 1984; Michael 1985), may form the remaining fifth of the cells but are thus certainly not the only neurons involved in color processing. In contrast to the lack of relationship between cone opponency and orientation selectivity, cone opponency and direction selectivity appear to be mutually exclusive. The cone-opponent neurons are much more sustained than nonopponent neurons. This suggests a dominant input coming from sustained LGN X-cells, but the higher temporal cut-off frequency and shorter latencies of opponent neurons do not confirm this idea.

The mixture $\eta$ to which a nonopponent cell does not respond allows us to approximate the relative weight of the two cone contributions. The average estimated S-cone weight is about a fifth. In the optic nerve of the gray squirrel, only 6% of the fibers receive input from the S-cone (Blakeslee et al. 1988). This small fraction of fibers has a large impact in the cortex, where four of five of the cells respond to the S-cone isolating stimulus.

We grouped cells which showed band-pass spatial frequency tuning for both the luminance contrast and the color gratings. These “band-pass cells” are much more selective for orientation but not for direction than low-pass cells.

The spatial frequency tuning for equiluminant color gratings is significantly lower than that for black-and-white gratings. We believe the shift is specifically caused by the equiluminant character of the stimulus and not due to a low luminance contrast. There may have been some luminance contrast in our equiluminant stimulus, but at least in other species, the spatial frequency tuning is largely invariant to changes in contrast (Albrecht and Hamilton 1982; Sceniak et al. 2002).

The range of S-M color sensitivity (the maximum response to an equiluminant grating divided by the maximum response to a contrast luminance grating) in the gray squirrel is strikingly different and much closer to unity than the range of L-M color sensitivity of macaque V1. Three-quarters of our cells have color sensitivities between 0.5 and 2, whereas only 30% of cells tested for L-M color sensitivity in the macaque are in this category (Johnson et al. 2001). In gray squirrel, only 1 of 154 cells tested responded more than twice as high to the equiluminant stimulus than to the luminance stimulus. Color sensitivity is not correlated to any other of the discussed cell properties except for a small positive correlation to the spatial frequency bandwidth measured with black-and-white gratings.

In the macaque, L-M equiluminant stimuli do not shift the spatial frequency tuning to lower frequencies. There is much evidence that, at least at the retinal and geniculate level, the L-M color system is distinct from the S-M color system. Therefore a more relevant comparison would be with the S-M color sensitivity in the monkey. Unfortunately, these data are not yet available. The relative weight of the S cone in macaque
V1 has been measured and is much smaller than in gray squirrel (mean: 0.05 vs. 0.22) (Johnson et al. 2004).

Cytochrome oxidase blobs similar to those seen in primate species, which have been implicated in color processing, are not apparent in gray squirrel V1 (Moore 2001). We did not find any evidence of clustering of color processing as non-cone-opponent and cone-opponent were mingled on each penetration. We cannot exclude the existence of a sparse mosaic of clusters with more or less cone opponency, nor can we exclude clustering with a spatial scale of <40 μm, which was the minimum distance between two cells on a single penetration.

**Laminar organization**

The clearest signs of laminar organization of information processing can be seen in the layer specificity of the response latency, Fig. 6, A and B. Layer 4, receiving the bulk of the input from the LGN (Harting and Huerta 1983), has a homogeneous short latency of ~26 ms after stimulus onset. Subsequently cells in layers 5, 6, and, finally, layers 2/3 respond. There are a few short-latency cells scattered around the bottom of layers 5 and 6. These may be cells receiving direct geniculate input as the LGN also projects to the dense layer of cells at the top of layer 6 (Robson and Hall 1975). The slower average response in layer 6 is consistent with the anatomical indications that the layer 4 geniculocortical projection is much stronger than the layer 6 projection.

In macaque (M. mulatta and fascicularis, Gur et al. 2004; Hubel and Wiesel 1968; M. nemestrina, Bullier and Henry 1980), ferret (Chapman and Stryker 1993), and to a lesser extent also in cat (Gilbert 1977; Henry et al. 1979; Jacob et al. 2003), there are reports that the layer receiving most of the thalamic projections is less tuned for orientation than the upper layers. A prevalence of unoriented cells in layer 4 has also been reported in mouse (Mangini and Pearlman 1980; Metin et al. 1998) and tree shrew (Chisum et al. 2003; Humphrey and Norton 1980). This trend is present but not significant in other macaque species (M. fascicularis) (Ringach et al. 2002) and in the owl monkey (Aotus trivirgatus) (O’Keefe et al. 1998) and is not detectable in the bush baby (DeBruyn et al. 1993). In other studies, not layer 4 but the deeper layers have cells with a broader orientation tuning than the superficial layers (mouse, Drager 1975; rat, Girman et al. 1999; hamster, Tiao and Blakemore 1976; macaque, M. fascicularis, Schiller et al. 1976b) (see Table 2). In gray squirrel, the orientation tuning does not vary significantly by layer. If anything, there is a lack of cells with narrow tuning widths in the lower region of layer 3. In gray squirrel, layer 4 does stand out by the relative absence of complex cells, see Fig. 2, A and B. Together with layer 6, the two layers receiving dense innervation from the LGN have a higher $F_4/F_0$ ratio (when measured with drifting or stationary gratings). The relative abundance of simple cells in the layers receiving thalamic input had also been noted in cats (Gilbert 1977; Gilbert and Wiesel 1979; Henry et al. 1979; Hubel and Wiesel 1962; Martin and Whitteridge 1984; Van Essen and Kelly 1973) and macaques (Hawken et al. 1988; Hubel and Wiesel 1968; Ringach et al. 2002; Schiller et al. 1976a). Some studies in the macaque find a higher proportion of simple cells particularly in layers 4B, 4C, α, and 6, which are layers receiving input from the magnocellular layers of the LGN (Bullier and Henry 1980; Kagan et al. 2002; Livingstone and Hubel 1984). One study also finds a higher proportion of simple cells in layer 4 of rat V1 (Parnavelas et al. 1983). Our results confirm that a higher proportion of simple cells in the thalamic input layers is a general rule. The linearity measure taken with static gratings shows this laminar distribution more clearly than the relative modulation to drifting gratings. In the macaque, the pattern is also less pronounced when the classification is made based on $F_1/F_0$ than when made based on subfield segregation (Kagan et al. 2002).

Our data suggest a very specialized role for layer 6, which has virtually no cone opponency and has a near monopoly on direction-selective cells. The same specialization in direction selectivity, though less pronounced, is present in cat (Gilbert 1977), bush baby (DeBruyn et al. 1993), and macaque (Orban et al. 1998). In these two primate species, cells around the layer 3/4 border (4B, 4C) are also more direction-selective (Snodderly and Gur 1995; but Kagan et al. 2002 also find direction-selective cells in upper layers). Thalamic input into this region is dominated by axons from the magnocellular LGN layers (Bullier and Henry 1980; Florence and Casagrande 1987). Layer 6 receives input from both X and Y cells in the LGN in the cat (Bullier and Henry 1979; Humphrey et al. 1985). Layer 6 also receives direct thalamic input in the gray squirrel, including input from the Y/W layers (Harting and Huerta 1983). This is consistent with the belief that gray squirrel Y cells are homologous to primate magnocellular cells (Van Hooser et al. 2003), but a smaller projection from LGN X-cell layers to layer 6 also seems to exist (Harting and Huerta 1983).

There are many sustained cells present in the layer 4/5 border region. The latency at this depth is slightly longer than in the rest of layer 4. Both observations suggest that the layer 4/5 border is more strongly driven by the sustained and slower Xcells of LGN layers 1 and 2 or the slow LGN W cells than by the faster transient Y cells from LGN layer 3. Adding to this suggestion, is that at the same cortical depth, cone opponency is higher than it is at all other depths.

**Summary**

We conclude that gray squirrel V1 has many properties in common with V1 of larger mammals and smaller, nonvisual mammals. The optimal spatial frequencies lie between those of rat and cat, as expected from behavioral measurements. Its high temporal frequency tuning sets gray squirrel apart from nocturnal rodents and places it among other diurnal animals. Gray squirrel has fewer oriented cells in V1 than the rat, an animal half its size. The macaque, another animal with cone-dominated vision, also has fewer oriented cells in V1 than smaller nocturnal primates. As in other animals, gray squirrel V1 possesses oriented simple and complex cells and unoriented cells. In Table 2, we have summarized several features of the organization of response characteristics that are common among different mammals or in which gray squirrel differs. Except for a bimodality in the modulation ratio, possibly separating simple from complex cells, there are no distinct cell class boundaries based on response properties. We find a range of different sensitivities to the two gray squirrel cone types. Cone opponency and direction selectivity are mutually exclusive. Cone opponency is absent in LGN-input recipient layer 6. This layer contains almost all direction-selective cells. The lack of laminar structure in direction selectivity seen in the
mouse and rat is therefore not common to all rodents. The response of cells in thalamic input layers 4 and 6 are more linear than in other layers. This is a consistent feature across species. Unlike other species, gray squirrel shows no reduced orientation selectivity in layer 4 or its infragranular layers.

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