Spatial phase sensitivity of complex cells in primary visual cortex depends on stimulus contrast

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The majority of visually responsive neurons in cat and monkey primary visual cortex are orientation selective (e.g., Ferster and Miller 2000). The widths of the orientation tuning functions are invariant to changes in stimulus contrast (Sclar and Freeman 1982; Skottun et al. 1987), but receptive field (RF) size and spatial frequency selectivity are contrast dependent (Sceniak et al. 1999, 2002). Contrast sensitivity is also influenced by prior stimulus history, such that sensitivity is dynamically enhanced at the prevailing stimulus contrast and reduced at low contrasts (Crowder et al. 2008; Hietanen et al. 2007; Ohzawa 1985). Another important characteristic of RF properties in the visual system; cat cortex; area 17; complex cell cortex of every species studied so far is that cells can be split into two distinct categories: simple and complex cells (e.g., cat and monkey: Hubel and Wiesel 1962; Skottun et al. 1991; marsupials: Ibbotson et al. 2005; rodents: Van Hooser et al. 2005). Simple cells have segregated RFs made up of oriented patches that detect either luminance increments (ON) or decrements (OFF) (Hubel and Wiesel 1962). This gives them the capacity to code the location of oriented edges in an image, i.e., the cells are sensitive to spatial phase. Complex cells show a range of RF characteristics, some with moderate segmentation of ON and OFF regions and others with no evidence of segregation. The mechanisms underlying complex cell receptive field structure involves a range of spatial phase sensitivities, from moderate to almost complete phase invariance. Recent studies using drifting sinusoidal gratings have shown that some complex cells increase their phase sensitivity as contrast is reduced (cat: Crowder et al. 2007; van Kleef et al. 2010; monkey: Cloherty and Ibbotson 2015; Henry and Hawken 2013). In cat, Crowder et al. (2007) also revealed that exposure to an adapting stimulus also leads to increased phase sensitivity. Despite this, studies have revealed that reduced contrast leads to increased phase sensitivity have all used drifting sine-wave gratings. As analyses of the responses to drifting gratings are unable to separate the effects of spatial and temporal phase summation, it has been difficult to establish the origin of the contrast-dependent phase sensitivity. Here we measured the phase sensitivity of neurons in cat primary visual cortex at multiple stimulus contrasts, using temporally modulated but stationary gratings presented at many different spatial phases within each cell’s RF. We show that there is a trend toward increased spatial phase sensitivity in the supragranular layers of cortex as contrast is reduced. Conversely, temporal phase sensitivity shows no consistent change as contrast is reduced.

METHODS

Surgical procedures. Extracellular recordings were made from single units in primary visual cortex (areas 17 and 18) of anesthetized cats (n = 4; 2.5 kg) (also see Crowder et al. 2007). Experiments were performed according to the National Health and Medical Research Council’s Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. All procedures were approved by the Animal Experimentation Ethics Committee at the Australian National
were quantitatively measured in a standard bank of preliminary tests. The depth of anesthesia was determined by an absence of the toe pinch reflex. Once anesthetized the animals were intubated, to ensure that the airways were kept open, and the right cephalic vein was cannulated. Animals were then placed in a stereotaxic frame, and anesthesia was maintained with gaseous halothane (1–2% during surgery, 0.5% during unit recordings) in a 2:1 mixture of N2O and O2. The animals were continuously monitored to ensure adequate levels of anesthesia by recording the electrocardiogram (ECG), the electroencephalogram (EEG), and end-tidal CO2 concentration. Blood pressure was measured noninvasively every 15 min. Any changes in the physiological indicators (ECG, EEG, blood pressure, or expired CO2) that may have suggested a changed level of anesthesia were mitigated by immediately increasing the concentration of inhaled halothane. Animals received a continuous intravenous infusion of fluids (2.5 ml·kg⁻¹·h⁻¹) containing Hartmann’s (lactated Ringer) solution (25% by volume), 5% glucose-0.9% NaCl solution (25% by volume), and an amino acid solution (50% by volume). Body temperature was maintained at 38.5°C by way of an electric heating blanket under feedback control. To prevent eye movements, a continuous intravenous infusion of gallamine triethiodide (Sigma, St. Louis, MO) was provided through the infusion line at a rate of 10 mg·kg⁻¹·h⁻¹. Animals were mechanically ventilated to maintain end-tidal CO2 between 3.5% and 4.5%.

Topical application of ophthalmic atropine sulfate (1%) and phenylephrine hydrochloride (10%) was used to dilate the pupils and retract the nictitating membranes. Neutral-power rigid gas-permeable contact lenses were fitted to the eyes to ensure corneal perfusion, and corrective lenses were placed in front of the eyes to focus the stimulus on the retina. Three-millimeter-diameter artificial pupils were placed between the eyes and the corrective lenses to minimize spherical and chromatic aberrations. Daily injections were administered to reduce secretions (atropine, 0.2 mg/kg sc), cerebral edema (dexamethasone), and an amino acid solution (50% by volume). Body temperature was maintained at 38.5°C by way of an electric heating blanket under feedback control. To prevent eye movements, a continuous intravenous infusion of gallamine triethiodide (Sigma, St. Louis, MO) was provided through the infusion line at a rate of 10 mg·kg⁻¹·h⁻¹. Animals were mechanically ventilated to maintain end-tidal CO2 between 3.5% and 4.5%.

To initiate anesthesia, animals received an intramuscular injection of ketamine hydrochloride (20 mg/kg im) and xylazine (1 mg/kg). The depth of anesthesia was determined by an absence of the toe pinch reflex. Once anesthetized the animals were intubated, to ensure that the airways were kept open, and the right cephalic vein was cannulated. Animals were then placed in a stereotaxic frame, and anesthesia was maintained with gaseous halothane (1–2% during surgery, 0.5% during unit recordings) in a 2:1 mixture of N2O and O2. The animals were continuously monitored to ensure adequate levels of anesthesia by recording the electrocardiogram (ECG), the electroencephalogram (EEG), and end-tidal CO2 concentration. Blood pressure was measured noninvasively every 15 min. Any changes in the physiological indicators (ECG, EEG, blood pressure, or expired CO2) that may have suggested a changed level of anesthesia were mitigated by immediately increasing the concentration of inhaled halothane. Animals received a continuous intravenous infusion of fluids (2.5 ml·kg⁻¹·h⁻¹) containing Hartmann’s (lactated Ringer) solution (25% by volume), 5% glucose-0.9% NaCl solution (25% by volume), and an amino acid solution (50% by volume). Body temperature was maintained at 38.5°C by way of an electric heating blanket under feedback control. To prevent eye movements, a continuous intravenous infusion of gallamine triethiodide (Sigma, St. Louis, MO) was provided through the infusion line at a rate of 10 mg·kg⁻¹·h⁻¹. Animals were mechanically ventilated to maintain end-tidal CO2 between 3.5% and 4.5%.

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Experiments were terminated by intravenous injection of an overdose of barbiturate (pentobarbital sodium, 150 mg/kg), after which animals were immediately perfused through the heart with 0.9% saline followed by 10% formal saline. The brain was then extracted for histological reconstruction of recording track locations (for details see Crowder et al. 2006).

**Recordings and visual stimulation.** To allow access to brain areas 17 and 18, a craniotomy was performed 0–8 mm posterior to interaural zero and 2–8 mm lateral to the midline. We recorded with gold- or platinum-tipped, lacquer-coated tungsten microelectrodes (FHC, Bowdoin, ME). Extracellular potentials were amplified, band-pass filtered (300 Hz–5 kHz) and then sampled at 40 kHz with a CED1401 interface and Spike2 software (Cambridge Electronic Design, Cambridge, UK). Neurons were isolated based on the shape and consistency of the extracellular spike waveform. Once a cell’s spike waveform was identified, its dominant eye and RF location were measured with hand-driven bright or dark bars projected onto a tangent screen. The nondominant eye was covered, and all further testing used the dominant eye. Visual stimuli were generated with a ViSaGe visual stimulus generator (Cambridge Research Systems, Cambridge, UK) via a video output of a computer-generated CRT monitor (Clinton monoray, 1,024 × 768 pixels, refresh rate 100 Hz) at a viewing distance of 57 cm. Drifting sine-wave gratings were presented in a circular aperture against a uniform background matched to the mean luminance of the gratings (57 cd/m²). Tuning functions for all neurons were quantitatively measured in a standard bank of preliminary tests. Parameters tested were preferred direction, spatial frequency, temporal frequency, RF size, exact RF location, and phase sensitivity, measured as described below. These tests were performed with drifting sine-wave gratings of 100% Michelson contrast. The RFs of all neurons analyzed here were located within 10° of the area centralis.

**Spike sorting** was performed off-line with Spike2 (Cambridge Electronic Design). The raw data were first high-pass filtered and candidate spikes identified as crossings of a manually adjusted threshold. Typically, we started with a very conservative threshold that detected only the largest-amplitude spikes and progressively lowered the threshold to detect smaller spikes, where present. Spike waveform templates were generated with the default parameters in Spike2 and each candidate spike compared against the templates and assigned to the template with the closest match. Spike2 uses a threshold crossing to trigger template creation. At least 8 spikes had to occur with a frequency higher than 1 in 50 spikes crossing the threshold to be considered to create a new template. New templates were created with a width of 32% of the amplitude of each sampling point of the initial seed spike. Spikes were considered for each template in order of decreasing maximum amplitude. At least 60% of the spike had to fall within the template that covered the time range beginning from spike onset and ending after the refractory period (afterhyperpolarization). Similar templates were then combined manually with the cluster parameters analysis in Spike2. The resulting templates were further refined by examining tuning curves for stimulus orientation and spatial and temporal frequency, calculated from the spikes matching each template. Templates that were judged both to have similar shapes and yield similar tuning curves were combined. A “merged template” was defined as the mean waveform over all spikes in the combined pool, and the correlation coefficient between it and the spikes in the combined pool was calculated. The mean correlation coefficient over spikes in each combined pool varied from a minimum of 0.73 to a maximum of 0.93 (population mean of 0.82). The standard deviation of the correlation coefficient across individual spikes in a combined pool varied from 0.07 up to 0.19 across cells (population mean of 0.13). Templates that gave a spontaneous rate that was not at least five times smaller than the peak response in the tuning curves were rejected as noise.

We quantified the quality of our spike sorting by calculating, for each cell, a signal-to-noise ratio (SNR) defined as the ratio of the amplitude of the average waveform to the standard deviation of the noise (Suner et al. 2005). The mean SNR over all cells in our population (n = 48) was 2.8, with 2 cells rated as “good,” 38 cells “fair,” and 8 cells “poor” according to the criteria of Suner et al. (2005). There was no substantive difference in our results or conclusions when the eight cells rated as poor were excluded. We therefore present the data for all 48 cells.

**Analysis of responses to contrast-reversing gratings.** For each cell presented, we measured the spiking activity to sinusoidally modulated contrast-reversing gratings that were presented at eight different spatial phases (0°, 45°, 90°, 135°, 180°, 225°, 270°, and 315°). Twelve different peak contrast levels were tested for every phase (1%, 2%, 4%, 6%, 8%, 12%, 16%, 24%, 32%, 48%, 64%, and 100% contrast). Gratings with different contrasts were presented for 0.5 s with stationary peak contrast, 1 s with sinusoidally modulated contrast, and another 0.5 s with stationary peak contrast. The choice of a stationary grating at peak contrast for the prestimulus condition allowed a continuous transition to the central, main stimulus not only for contrast-reversing gratings but also for drifting gratings, which were also presented to allow comparison to previous results using drifting gratings (see below). In some experiments these periods were chosen to be 1 s, 2 s, and 1 s, respectively, to allow the collection of longer sequences of modulated contrast.

To analyze the combined spatial and temporal tuning properties of each cell, two-dimensional spatio-temporal period histograms were constructed from the spiking response to the contrast-reversing portion of the stimulus. This involved assigning each spike to 1 of 64 bins...
in an 8 × 8 grid corresponding to the combinations of 8 spatial and 8 temporal phases (0°, 45°, 90°, 135°, 180°, 225°, 270°, and 315° in each case; see, e.g., Fig. 1). By plotting these histograms over the range of contrasts presented, any changes in the spatial or temporal tuning of the cell with contrast could be visualized.

To quantitatively summarize the spatio-temporal tuning of cells and how this changed with contrast, the period histograms were smoothed by fitting to an appropriate function, and the smoothed version was used to extract a variety of tuning measures. As the great majority of period histograms appeared bimodal, we fit a sum of two von Mises distributions (the von Mises distribution is a unimodal distribution for periodic variables, such as phase, analogous to the Gaussian distribution for nonperiodic variables):

\[ P(\varphi_s, \varphi_t) = \sum_{n=1}^{2} B_n \frac{e^{K_n \cos(\varphi_s - \hat{\varphi}_{s,n})} + e^{K_n \cos(\varphi_t - \hat{\varphi}_{t,n})}}{4\pi I_0(K_n)} \]

where \( \varphi_s \) and \( \varphi_t \) are variables for the temporal and spatial phases; \( \hat{\varphi}_{s,n} \) and \( \hat{\varphi}_{t,n} \) are the corresponding peak (or preferred) phases for the two modes \( n = 1, 2; K_{s,n} \) and \( K_{t,n} \) are parameters that are inversely related to the temporal or spatial width of the distribution of each mode \( n \); and \( B_n \) is the total number of spikes belonging to mode \( n \) (across all spatial and temporal phases). The parameters \( \hat{\varphi} \) and \( 1/K \) are analogous to the mean and variance of a Gaussian distribution, and \( B \) is the area under the distribution. \( I_0 \) is the modified Bessel function of the first kind of order 0 and is required in the denominator to normalize the integral of the distribution.

The measures extracted from this fitted function to characterize each cell’s tuning were the preferred spatial and temporal phases, \( \hat{\varphi}_{s,n} \) and \( \hat{\varphi}_{t,n} \), the peak spike rate \( A_n \) of each mode (\( A_n = \frac{B_n}{4\pi I_0(K_{s,n}) I_0(K_{t,n})} \)), where \( T \) is the duration of the temporal phase bins, which varied depending on the cell’s optimal temporal frequency), and the spatial and temporal modulation depths, \( D_{s,n} \) and \( D_{t,n} \). These latter quantities are defined as the relative change in spike rate between the peak of each mode and an appropriately defined trough; for example, \( D_{s,1} \) is the relative change in spike rate from the peak of mode 1, at \( (\hat{\varphi}_{s,1}, \hat{\varphi}_{t,1}) \), to the trough at \( (\hat{\varphi}_{s,1}, \hat{\varphi}_{t,1}) \), as \( \varphi_s \) is varied, keeping \( \varphi_t \) fixed at its peak value \( \hat{\varphi}_{t,1} \) (see Fig. 2 for a graphical illustration). \( D_{s,n}, D_{t,n} \) etc. are defined analogously. The relative change is referenced to the peak rate and lies between 0 and 1.

The fitting was performed by a least-squares optimization on the parameters \( B_n, \hat{\varphi}_{s,n}, \hat{\varphi}_{t,n}, K_{s,n}, \) and \( K_{t,n} \) using the lsqnonlin function in MATLAB (The MathWorks, Natick, MA), which employs the trust region reflective algorithm. As the period histogram at 100% contrast typically had the greatest SNR, this was fit first with random initial estimates of the parameters provided to lsqnonlin. The best fit over 100 runs with random initial parameter estimates was used to avoid fits that were only local optima. For lower contrast levels, the best parameter estimate for the next highest contrast was used as the estimate to initialize lsqnonlin. This process allowed us to continuously trace the parameters corresponding to each distinct mode across stimulus contrasts. For some cells, one of the two modes became indistinct at low contrasts. To avoid fitting the second peak to noise in this case, the bimodal fit was rejected if the number of spikes \( B_n \) belonging to the smaller mode was <10% of the total. In this case the period histogram was refit with a unimodal von Mises distribution.

In addition, to gauge the statistical significance of the parameter estimates the following threefold analysis was performed for each cell. First, to ensure that we were measuring reliable stimulus-evoked spiking, for each contrast we excluded any parameter estimates for which the mean spike rate across the period histogram was not above the mean spontaneous rate by at least twice the sum of both standard errors. Second, a standard error for each of the fitted parameters was calculated by refitting the von Mises distribution to period histograms obtained from each single repetition of the stimulus \( n = 10 \) or 20 instead of the pooled data. The resulting set of parameter estimates, across repetitions, were then used to calculate a standard error of the mean. Third, some parameters, such as modulation depth and peak spike rate, were subject to biased estimates when the total number of spikes used to estimate them was low (i.e., the mean value of the parameter estimate using a subsample of the data was systematically either above or below the parameter estimate obtained using all of the data). To determine whether any given parameter estimate was biased, Monte Carlo simulations were performed to examine how the number of spikes in the associated period histogram affected the parameter

**Fig. 1.** Response of an example simple cell (A and B) and an example complex cell (C and D) to a contrast-reversing grating stimulus presented at 100% peak contrast. A and C: spike rastergrams from 10 repeats of the stimulus at each of 8 spatial phases, delimited by the horizontal faint gray lines. Vertical bold gray lines separate 4 consecutive cycles of the stimulus, and vertical faint gray lines delimit 8 temporal phases within each period. B and D: period histograms corresponding to the rastergrams, formed by pooling spike responses over all cycles of the stimulus; white indicates the bin(s) with maximal spike count in the histogram and black a zero spike count.
estimate. A given number of spikes ($N$) were randomly sampled from
the period histogram and used to reestimate the parameters as de-
scribed above. This was repeated 100 times, with independent resa-
mping to obtain estimates for the mean and standard deviation of the
parameter estimates as a function of the number of spikes, $N$, ranging
from 64 to 65,536 in powers of 2. This range was chosen to span the
number of recorded spikes, $N_{\text{exp}}$ in the experimentally obtained
histograms, which ranged from $\sim 100$ to $>10,000$ depending on cell
and stimulus contrast. For the great majority of parameters it was
found that the simulated parameter estimate converged to the exper-
imentally obtained estimate at large spike counts, but in some cases a
systematic discrepancy between the two estimates was observed at
low spike counts. The experimentally obtained parameter estimate
was deemed unbiased if it differed from the simulated parameter
estimate with $N = N_{\text{exp}}$ simulated spikes by less than two standard
errors of its mean.

An analysis of responses to drifting gratings. The population of cells
that were presented with contrast-reversing gratings were also pre-
sented with drifting sinusoidal gratings and formed a subset of a much
larger population from previous studies that were only presented with
drifting gratings. For these cells we performed an analysis similar to
that described above to obtain the modulation depth as the spatio-
temporal phase was advanced to produce the traveling wave charac-
teristic of these stimuli. The direction and spatial and temporal
frequency of the drifting gratings were chosen to be optimal for the
cell and were presented within a circular aperture of optimal size
centered on its RF. Spiking activity was recorded for the same 12
different peak contrast levels used for contrast-reversing gratings (i.e.,
1%, 2%, 4%, 6%, 8%, 12%, 16%, 24%, 32%, 48%, 64%, and 100%
contrast), presented in randomized order.

Similar to the analysis for contrast-reversing gratings, the response
was first summarized with a one-dimensional period histogram at
eight spatio-temporal phases ($\phi_s = 0^\circ, 45^\circ, 90^\circ, 135^\circ, 180^\circ,
225^\circ, 270^\circ, 315^\circ$ in each case). This was fit to a single one-dimensional
von Mises distribution:

$$P(\phi_s) = B e^{K_s \cos (\phi_s - \phi_e)}$$

where $B$, $K_s$, and $\phi_e$ are defined analogously to the two-dimensional
case. The spatio-temporal modulation depth, $D_s$, was calculated from
this fit as the ratio of the change in amplitude between the peak and
the trough relative to the peak amplitude. The fitting procedure and
statistical analysis were analogous to those used for contrast-reversing
gratings.

The modulation ratio ($F_1/F_0$) was calculated for each cell
presented with drifting gratings by performing a fast Fourier
transform of the one-dimensional period histogram described
above (fft function, MATLAB) and dividing the magnitudes of the
appropriate components.

Histology. Brains were sectioned in the coronal plane by cryosec-
tioning with a section width of 24 $\mu$m. Slices were mounted on subbed
slides and stained with thionin for Nissl substance (Crowder et al.
2006; Price et al. 2006). Images of slices were digitized using 1–4
megapixel cameras attached to a microscope with $\times 4$ magnification.
Cortical layer boundaries for all sections were marked with standard
indicators such as cell size and density (Payne and Peters 2002). The
sections were then aligned using the surface and gray/white matter
boundaries with the use of AMIRA (FEI) to create three-dimensional
reconstructions with embedded layer boundaries and electrode tracks.
From the models, the recording locations could be determined in
depth and horizontal extent relative to the brain surface. Specific
lesion markers in nearby tracks were placed after the recording period
had finished with specific lesion electrodes. These gave verifiable
markers to compare recording depths with the brain surface. Based on
the thionin stain, we were able to locate the boundaries of layers 1, 4,
5, and 6 with great accuracy. The boundary between layers 2 and 3
was far more difficult to determine in some sections. Therefore, when
layers 2 and 3 are discussed separately below, the boundary between
the two is most often taken as the line that is exactly halfway between
the layer 1/2 and 3/4 borders. In general, the results presented from
layers 2 and 3 do not differ, but we segregated the data using the
halfway point in case any systematic differences were evident.

RESULTS

Response to contrast-reversing gratings. We recorded from 48 cells in primary visual cortex presented with contrast-
reversing gratings at their preferred orientation and spatial and
temporal frequency. We deliberately targeted complex cells
(classified on the basis of $F_1/F_0$ of their response to optimal
drifting gratings at 100% contrast), of which there were 42 in
our sample. Six simple cells were accepted for full analysis to
give us a comparison with the complex cell population.

The raster plot in Fig. 1A shows responses from a simple cell
presented with contrast-reversing gratings with a peak lumin-
nance contrast of 100%. The different spatial phases are
arranged along the y-axis in Fig. 1A delimited by horizontal

![Fig. 2. Illustration of the procedure for extracting the peak phase and spatial and temporal modulation depth from period histograms for the simple (A and B) and complex (C and D) cells introduced in Fig. 1. Raw period histograms in A and C are first smoothed by fitting to a double-peak von Mises distribution (see METHODS) to give the histograms in B and D, respectively. For each peak (large circles or triangles) the corresponding spatial and temporal phases are extracted, $\phi_s$ and $\phi_t$. The spatial modulation depth of each peak, $D_s$, is obtained along the cross sections through the peak indicated by the overlaid vertical line connecting smaller symbols (circles or triangles). The temporal modulation depth is the change between peak and trough response, relative to the peak. Similarly, the temporal modulation depth, $D_t$, of each peak is obtained from the horizontal cross sections with the appropriate symbol. The peak spike rate is the spike rate at each peak.](http://jn.physiology.org/ by 10.220.33.4 on September 22, 2016)
thin gray lines; vertical thick gray lines delimit consecutive cycles during the sinusoidal modulation of the grating contrast, and vertical thin gray lines delimit different temporal phases within each cycle. The corresponding spatio-temporal period histogram obtained by pooling the data from the raster plot across cycles and trials is shown in Fig. 1B. As is typical of simple cells, at two spatial phases ($\varphi_s = 45^\circ$ and $45^\circ + 180^\circ$) the response is small. At a spatial phase of $\varphi_s = 135^\circ$ and a temporal phase of $\varphi_t = 45^\circ$, there is a robust peak in response that clearly demonstrates a half-wave rectification. A second similar response peak occurs at a spatial phase of $\varphi_s = 135^\circ + 180^\circ$, but this peak is displaced in time to $\varphi_t = 45^\circ + 180^\circ$, reflecting the $180^\circ$ shift in the spatial phase of the grating. This typical simple cell response acts as a baseline for comparison with complex cell behavior. The key features of the simple cell response are the clear spatial and temporal null phases, where responses are small (black). When the grating is spatially “in phase,” the temporal form of the response is a half-wave rectified version of the sinusoidally modulated input. This results in two response peaks separated from each other by $180^\circ$ in space and time by deep troughs with little or no response.

Figure 1, C and D, show the response of a complex cell to the same contrast-reversing grating stimulus. The raster and period histograms reveal similar response amplitudes at all spatial phases and a frequency-doubled temporal response, resulting from full-wave rectification of the sinusoidally modulated input. As for the simple cell, two response peaks are evident; however, in this case they show little spatial modulation, while in the temporal domain the peaks are again separated by $180^\circ$ but with shallower intervening troughs (gray rather than black).

To quantify these space-time tuning characteristics at a range of stimulus contrasts, the modulation depth and phase separation of the two response peaks were calculated from the period histograms in both spatial and temporal dimensions. This process is illustrated in Fig. 2 (for the example simple and complex cells shown in Fig. 1 at 100% contrast). The histo-

Fig. 3. Change in response measure for the example complex cell shown in Fig. 1. A: raw period histograms as a function of contrast level. B: corresponding smoothed versions. From these the following measures are extracted at each contrast level: spatial modulation depth (C), temporal modulation depth (D), peak spatial phase (E), peak temporal phase (F), peak spike rate (G). At each contrast, measures from both peaks are shown (circles or triangles), except when only a single peak could be fitted and/or the response was not significantly driven and/or the measure was assessed to be biased (see METHODS) (gray). H: mean spike rate across all stimuli, with the horizontal black line indicating the spontaneous rate. Error bars show ± 1 SE but are not given on gray data points.
grams were first smoothed by fitting them to double-peaked, two-dimensional “periodic-Gaussian” functions (von Mises distributions; mean $R^2$ of 0.61 over all cells and contrast levels): this allowed the two main peaks to be identified that account for most of the response variation (see METHODS). For each peak (denoted by large circle/triangle symbols in Fig. 2) we calculated the spatial and temporal modulation depths, $D_s$ and $D_t$, the peak phases, $\hat{\phi}_s$ and $\hat{\phi}_t$, and the peak response rate as illustrated in Fig. 2 (see METHODS). The modulation depth measures the relative change in response from peak to trough along a given dimension and varies from 0 for a completely unmodulated response to 1 for a completely modulated response. The phase separation of the peaks in the spatial and temporal dimensions, $\Delta\hat{\phi}_s$ and $\Delta\hat{\phi}_t$, was calculated as the difference in the peak phases and varies between 0° and 180°.

Figure 3 summarizes the outcomes of this characterization of tuning properties as a function of contrast for the example complex cell shown in Fig. 1. The period histograms in Fig. 3A show the unfitted response of the example cell at each stimulus contrast tested, and Fig. 3B shows the corresponding fitted responses. The measured spatial modulation depth of both peaks (circles and triangles) tended to increase as contrast was reduced (Fig. 3C); it varied from a minimum of $D_s = 0.18 \pm 0.05$ at 100% contrast (mean across the 2 peaks ± SE) to a maximum of $D_s = 0.6 \pm 0.05$ at 6% contrast, which was just above the lowest contrast that drove the cell significantly above its spontaneous rate (see Fig. 3H). In comparison, the measured temporal modulation depth was generally high but showed no consistent change with contrast (Fig. 3D). The spatial phase separation of the response peaks was $88 \pm 23^\circ$ at 100% contrast but decreased as contrast was reduced (Fig. 3E). In comparison, the temporal phase separation remained consistently around 180° across all driving contrasts (Fig. 3F). An advance to earlier temporal phases at higher contrast is also evident in Fig. 3F, an observation that was typical of other cells in this study and consistent with previous reports (Albrecht 1995; Movshon et al. 1978a, 1978b). As expected, both the
peak (Fig. 3G) and overall mean (Fig. 3H) spike rate increased as a function of stimulus contrast.

For comparison, Fig. 4 shows the same analysis for the example simple cell shown in Fig. 1. In this case, both the spatial and temporal modulation depth were high but not completely saturated and did not change markedly with contrast level (Fig. 4, C and D). Similarly, both the spatial and temporal phase separation of the peaks were around 180° and did not change markedly with contrast (Fig. 4, E and F). As for the complex cell shown in Fig. 3, the mean and peak spike rate tended to increase with stimulus contrast (Fig. 4, G and H).

The mean modulation depth over the population of cells is shown in Fig. 5 as a function of contrast. Mean spatial modulation depth (squares) showed a general trend to greater modulation depth as contrast is reduced, with the increase being most pronounced from high contrast (48%, 64%, and 100%) to mid contrast (16%, 24%, 32%). In comparison, mean temporal modulation depth (diamonds) changed less with contrast, being greatest at mid contrasts and declining at both high (48%, 64%, and 100%) and low (6%, 8%, and 12%) contrasts.

The trends in the population means of modulation depth were indicative of overall population behavior but also masked considerable variability within the population. We therefore compared modulation depth at low, mid, and high contrasts (as defined above) on a cell-by-cell basis. This revealed that the trend to greater spatial modulation depth as contrast was reduced was common among our complex cell population. This is evident in Fig. 6A, which shows a scatterplot of modulation depth averaged across low contrasts vs. modulation depth averaged across high contrasts, excluding any contrast value that did not evoke significant driven activity. A far greater proportion of cells showed significantly greater spatial modulation depth at low than at high contrast (25 vs. 6, with 11 cells showing no significant change; black points below the diagonal line vs. black points above the diagonal; gray points are within 2 SEs of the diagonal and are not considered significantly different). These data are also presented as a histogram of the change in spatial modulation depth, ΔΔD_s, from low to high contrast (Fig. 6B). Over 44% of cells showed a significant increase in spatial modulation depth of 0.15 or more; the mean change in modulation depth over population was 0.08, which was significantly greater than zero (P < 10^-4, 1-sided Student’s t-test). This positive bias in the change in modulation depth did not depend significantly on the magnitude of the modulation, as seen by the (total least squares) linear regression analysis (Markovsky and van Huffel 2007) in Fig. 6A (dashed line), which showed a significant positive offset from the diagonal of 0.08 (P < 0.05, Student’s t-test), but the slope was not significantly different from unity (P > 0.05, Student’s t-test). (Note that while both the histogram and scatterplot give a population bias of 0.08 for the change in modulation depth, the significance level is less for the scatterplot because the model has two parameters, slope and intercept, instead of a single parameter, the mean, as for the histogram.)

Population data for temporal modulation depth is presented in the same format for low vs. high contrast (Fig. 6, C and D) and mid vs. high contrast (Fig. 6, E and F). While it was common for temporal modulation depth to change significantly from high to low contrast, by up to 0.25, there was no clear trend toward either an increase or a decrease across our population and the mean change in modulation depth over the population was not significantly different from zero (P > 0.05, 1-sample Student’s t-test). However, when mid contrasts were compared to high contrasts, there was a small bias to greater temporal modulation depth in the population, with a mean change in modulation depth over the population of 0.03, which was significantly greater than zero [albeit marginally at P < 0.05, 1-sided Student’s t-test with Bonferroni correction for multiple (k = 2) comparisons]. Linear regression fits to the scatterplots in Fig. 6, C and E, do not give offsets from the diagonal that are significantly different from zero or slopes significantly different from unity at P = 0.05 significance level (1-sided Student’s t-test).

While it was common for cells in the population to increase spatial modulation depth as contrast was reduced, this was not typically accompanied by a tendency for the spatial phase separation of the response peaks to approach 180° as would be expected for a simple cell. This is illustrated in Fig. 7A, which shows a histogram of spatial phase separation at low contrast for the population as a whole (gray) and for those cells showing a significant effect of increased spatial modulation depth from high to low contrast (black). The percentage of cells in these two categories with a spatial phase separation falling in the 180° bin was 23% and 20%, respectively, which was not significantly different from chance (20%, P > 0.05, binomial test). In comparison, >90% of cells had a temporal phase separation falling in the 180° bin at low contrast (Fig. 7B), which was highly significant (P < 10^-10, binomial test).

**Comparing responses to contrast-reversing and drifting gratings.** To relate the above results to previous observations in complex cells using grating stimuli that were drifting rather than reversing (Cloherty et al. 2015; Crowder et al. 2007; Henry and Hawken 2013; van Kleef et al. 2010), we recorded the responses of the same population of 48 cells to drifting gratings at the same spatial and temporal frequencies as used for the contrast-reversing gratings. Recall that the spatial phase of these stimuli advances steadily with time. For drifting gratings it is common to calculate the F1/F0 as a basis for classifying cells as either simple (F1/F0 >1) or complex

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Fig. 5. Change in population mean modulation depth as a function of contrasts that typically drove cells significantly above spontaneous rate (i.e., ≥6%). Squares show spatial modulation depth, and diamonds show temporal modulation depth. Error bars show SE.
this is the magnitude of the first- to the zeroth-order Fourier components of the response (Skottun et al. 1991). To allow comparison of these previous studies to our analysis of modulation depth for contrast-reversing gratings, described above, we first compared F1/F0 to a measure of modulation depth obtained from drifting gratings, $D_{st}$. We then compared this measure of modulation depth for drifting gratings to the measures of modulation depth we obtained for contrast-reversing gratings in the same population of cells.

This measure of modulation depth, $D_{st}$, was calculated for each cell and contrast level from a one-dimensional fitted period histogram of the spatio-temporal phase of the drifting grating, following a procedure analogous to that used for the contrast-reversing gratings. Briefly, this involved fitting a one-dimensional von Mises distribution to the response period histogram and using it to extract the modulation depth (see METHODS). A comparison of F1/F0 with $D_{st}$ at 100% contrast is shown in Fig. 8A and exhibits a high correlation between these two measures up to F1/F0 of ~0.7. This captures the great majority of our complex cells. In Fig. 8A the large black circles indicate those cells for which we also recorded responses to contrast-reversing gratings (“restricted population”; $n = 48$). These cells are consistent with the much larger population of cells for which we recorded responses only for drifting gratings ($n = 755$, small black dots; Fig. 8A).

The relationship between modulation depth to drifting gratings and the modulation depths obtained from contrast-reversing gratings in the restricted population of $n = 48$ cells is shown in Fig. 8, B and C, for 100% contrast. There is a moderate correlation between the modulation depth obtained from drifting gratings and the modulation depths obtained from contrast-reversing gratings in both spatial and temporal dimensions (Pearson’s correlation coefficient of 0.79 for $D_s$ vs. $D_{st}$ and 0.73 for $D_t$ vs. $D_{st}$). A total least-squares regression line for spatial vs. spatio-temporal modulation depth (Fig. 8B) gave an intercept that was not significantly different from zero and a slope that was not significantly different from unity (Student’s $t$-test, $P > 0.05$). This indicates that the degree of spatial modulation exhibited by a cell to reversing gratings is broadly consistent with its spatio-temporal modulation depth to drifting gratings, at 100% contrast. In comparison, the regression line fit to the temporal vs. spatio-temporal modulation depth (Fig. 8C) had both an intercept significantly greater than zero and a slope significantly less than 1 (Student’s $t$-test, $P < 10^{-4}$ and $P < 0.001$, respectively). This is consistent with the observation that contrast-reversing gratings always produced some
Next we examined the effect of reducing the contrast of the drifting gratings on the spatio-temporal modulation depth (Fig. 9). Similar to the results for spatial modulation depth with contrast-reversing gratings, when presented with drifting gratings there was an increase in modulation depth averaged across the larger population as contrast was reduced (Fig. 9A). A large proportion of cells significantly increased their mean spatio-temporal modulation depth at low (6%, 8%, and 12%) contrasts compared with high (48%, 64%, and 100%) contrasts. This was true for both the restricted (n = 48) and the larger (n = 755) populations: the ratio of the number of cells showing a “significant increase” to a “significant decrease” to “no significant change” was 18:8:22 and 264:87:404 for these two populations, respectively (here a significant change is defined as one exceeding 2 SEs from the mean). These data are shown for the restricted population in Fig. 9B, as a scatterplot of ΔDa at high vs. low contrast, and in Fig. 9C, as a histogram of the change in modulation depth from high to low contrast, ΔDa (cf. Fig. 6 for the contrast-reversing gratings). The mean change in modulation depth was 0.11 across the restricted population, which was significantly greater than zero (P < 0.001, 1-sided Student’s t-test). A total least-squares regression line fit to the data in Fig. 9B gave a significant positive bias in modulation depth of 0.13 for the offset from the diagonal (i.e., low compared with high contrast; P < 0.05, 1-sided Student’s t-test) and a slope that was not significantly different from unity (P > 0.05, 1-sample Student’s t-test). Data for the larger population of cells are shown in the same format in Fig. 9, D (scatterplot) and E (histogram) and are consistent with the results of the restricted population [Fig. 9D: significant positive bias in modulation depth of 0.05 (P < 0.001) and slope not significantly different from unity (P > 0.05); Fig. 9E: significant mean change in modulation depth of 0.09 (P < 10⁻¹¹); statistical tests as per restricted population].

Laminar profile of effect of contrast on modulation depth.

The laminar profile of the effect of contrast on spatial and temporal modulation depth for contrast-reversing gratings is shown in Fig. 10, A and B, respectively. The increase in spatial modulation depth, ΔDa, from low to high contrast was observed consistently in the supragranular layers (2/3), with 20/25 cells in these layers having a significant increase in modulation depth (ΔDa > 2 SEs of the mean above zero). In contrast, the population of cells in layers 4 and 5 showed both significant increases and significant decreases in modulation (ratio of significant increase to significant decrease to no significant change was 4:4:4 in layer 4 and 4:2:3 in layer 5). Consistent with this, the mean increase in spatial modulation depth across cells in these layers was significantly greater that zero (red diamonds, 0.14 ± 0.03 for layer 2 and 0.18 ± 0.04 for layer 3, mean ± 2 SEs; P < 0.001, 1-sided Student’s t-test). In contrast, neither layer 4 nor layer 5 had a significant change in mean spatial modulation depth (−0.02 ± 0.01 and 0.04 ± 0.03, respectively; P > 0.05, 1-sample Student’s t-test). For temporal modulation depth, the lack of any consistent effect of contrast level on the population as a whole was also observed in the laminar breakdown for layers 2, 4, and 5, whose mean change in temporal modulation depth, ΔDt, was not significantly different from zero (P > 0.05, one-sample Student’s t-test). However, layer 3 exhibited a significant increase in mean modulation depth (0.1 ± 0.02; P < 0.001, 1-sided Student’s t-test).

Recall that for this population of cells we also recorded responses to drifting gratings (n = 48). The laminar profile of ΔDa for drifting gratings was similar to the laminar profile of ΔDa for the contrast-reversing gratings: both showed that the effect of increased modulation at low vs. high contrast occurred most frequently in the supragranular layers (compare distributions indicated by black symbols in Fig. 10C with the distribution shown in Fig. 10A).

The laminar profile of ΔDa for the restricted population was also consistent with the laminar profile of the larger population. Figure 10D shows ΔDa for 427 cells from our larger population, plotted according to their laminar membership. These cells represent the subset of the larger population for which we had data for both drifting gratings and laminar membership but not reversing gratings. In Fig. 10D, black circles show the cells that exhibited a significant change in modulation depth and gray symbols show the cells that exhibited a nonsignificant change. The findings for the restricted and larger populations were highly consistent except in layer 4, where the mean value of ΔDa was significantly greater than zero in the larger population (P < 0.001, 1-sided Student’s t-test) but not so for the restricted population (P > 0.75).

DISCUSSION

Recent findings with drifting sinusoidal gratings have revealed that a large proportion of complex cells in the primary visual cortical areas of cat (areas 17 and 18) and monkey (V1) increase their phase sensitivity at low stimulus contrasts (Clo-
herty and Ibbotson 2015; Crowder et al. 2007; Henry and Hawken 2013; van Kleef et al. 2010). However, this effect is very rare in the second visual area (V2) in primates (Cloherty and Ibbotson 2015). These observations are interesting because they reveal that changes to the level of contrast-dependent feedforward drive to the primary visual cortex alters the phase sensitivities of the neurons, while further spatial integration in higher cortical areas (V2) appears to remove this property. The discovery of contrast-dependent phase sensitivity in the primary visual cortex adds to the list of contrast-related phenomena that influence cortical RFs (see introduction).

However, there is a problem with all of the work done to date. By analyzing the responses to drifting gratings it is possible to measure phase sensitivity, but the answers given combine the influences of spatial and temporal phase (Wiesel et al. 2001). In the present work we have used contrast-reversing gratings and compared the results with those from drifting gratings. Contrast-reversing gratings remain fixed in space but are modulated in time. Consequently, by testing at multiple spatial phases it is possible to assess the influence of the spatial and temporal components of the responses separately. In response to visual stimulation by contrast-reversing gratings, simple cells exhibit nearly linear spatial summation properties (Movshon et al. 1978a). That is, they are spatial phase (position) selective and their response is primarily a half-wave rectified version of the sinusoidal stimulus. Consequently, the response in most simple cells is nearly 100% modulated by the spatial phase of the grating. Conversely, in response to contrast-reversing gratings, complex cells exhibit spatial phase insensitivity and, in the time domain, show effects ranging from frequency doubling to near-complete phase insensitivity (Movshon et al. 1978b). In the temporal domain, frequency-doubled responses arise because all regions of the RF respond to brightness increments and decrements. As contrast changes twice per cycle with a contrast-reversing grating, a frequency-doubled response is expected.

Our novel finding is that a proportion of complex cells, which reside most frequently in the supragranular (and granular layers), have spatial phase sensitivity that is contrast dependent. At high contrast robust responses are generated regardless of the spatial phase of an optimally oriented, stationary grating, but at low contrast robust responses are generated only by gratings with specific spatial phases. This behavior is reminiscent of simple cells, which show a clear preference for a specific spatial phase (Movshon et al. 1978a, 1978b; Williams and Shapley 2007). However, the depth of response modulation with spatial phase in these complex cells at low contrast is generally not as great as for simple cells. Also, the spatial phase separation of their response peaks at low contrast is not typically 180° (as it is for simple cells) but rather varies from cell to cell with no obvious bias. These results are robust against the quality of the fit of the data to the von Mises function: there was no significant correlation of any of these
effect measures with quality of fit as quantified by the $R^2$ value (correlation coefficient < 0.1).

One possible explanation for the results presented here relates to the hierarchical model of complex cell RF formation, which posits that spatial phase invariance in complex cells arises from multiple simple subunits arranged to span several spatial phases (Hubel and Wiesel 1962). As contrast is reduced, the number of simple cell subunits that provide input to a given complex cell may be reduced because they no longer provide synaptic drive sufficient for the recorded cell to attain spiking threshold. The end result would be that one remaining simple subunit would provide the suprathreshold drive (van Kleef et al. 2010). As this one simple subunit has a distinct spatial phase preference, only stimulation close to that spatial phase will generate spiking responses at low contrast. This model is not well supported by the present data. If this were the case, we would expect complex-like response characteristics at high contrast (i.e., weak spatial phase sensitivity, small spatial modulation depth, and temporally full-wave rectified responses with peaks separated by 180° in time) but simple-like response characteristics at low contrast (i.e., high spatial phase sensitivity, large spatial modulation depth, and temporally half-wave rectified responses with peaks separated by 180° jointly in time and space). While the responses of some cells moved in this direction, this clear pattern was never observed in a categorical fashion. Notably, the spatial phase separation of the complex cell population at low contrast showed no tendency to cluster around a value of 180°, as occurs for simple cells. Furthermore, although the spatial modulation depths of the complex cell population at low contrast were frequently greater than at high contrast, they were rarely as great as the spatial modulation depths typical of simple cells.

These findings are consistent with a model in which one subregion of the complex cell’s excitatory RF (e.g., either an ON or OFF subregion) becomes dominant, leading to greater spatial phase sensitivity at low contrast when using both moving and contrast-reversing gratings. This suggestion does not contradict the earlier finding that the total area of the RF that generates spiking activity expands at low contrast (Sceniak et al. 1999). It is possible for the total excitatory area to increase while at the same time one subfield (either ON or OFF) exhibits greater relative gain. It has been known since the very first descriptions of complex cell RFs that some cells have discrete zones that are selective for increments (ON) or decrements (OFF) in luminance (Henry 1977; Hubel and Wiesel 1962; Mata and Ringach 2004). It might be that these subfields are accentuated or the separation between them is increased at low contrast. Durand et al. (2012) used briefly presented light and dark bars to map the spatial structure of RFs in monkey
primary visual cortex. They performed this mapping at a range of stimulus contrasts and found that reducing contrast significantly increased the separation between light and dark subregions in the RFs. These findings support our observations that reducing contrast leads to changes in the spatial modulation depths to contrast-reversing gratings of complex cells. It is possible that the observations presented here and in previous experiments (Cloherty and Ibbotson 2015; Crowder et al. 2007; Henry and Hawken 2013; van Kleef et al. 2010) relate to the fact that some complex cells have more uneven ON and OFF zones in their RFs, which are particularly sensitive to changes in contrast. Yeh et al. (2009) have shown in macaque cortex that dark responses dominate in layer 2/3 cells but not in layer 4 cells. In our observations, we noted that complex cells in cat cortex that showed strong shifts from phase invariant to phase sensitive were overrepresented in layers 2/3.

The segregation of cortical cells into simple and complex types, based on the relative modulation (F1/F0) of responses to moving gratings, may be as much to do with the nonlinearities of the spiking threshold as it is to do with the spatial structure of the RFs (Mechler and Ringach 2002; Priebe et al. 2004). It is therefore reasonable to ask whether the increase in modulation depth as contrast is reduced (Cloherty and Ibbotson 2015; Crowder et al. 2007; Henry and Hawken 2013; van Kleef et al. 2010) might be due to the nonlinear spiking behavior of the cells. If one assumes that the subthreshold response comprises a sum of components, one modulated by spatial phase and the other invariant to it, one can envisage a number of interactions that could give rise to reduced modulation depth at low contrast. For example, it could be that at low contrast only the peaks of the modulation exceed the spiking threshold (an “iceberg effect”), leading to large modulation depth, while at high contrast the invariant component of the response is driven above threshold so that the modulated component causes only a “ripple” on top of the unmodulated component. Another possibility is that the two components scale differently with contrast; for example, the modulated component scales linearly (like simple cells) while the invariant component scales quadratically (like the “energy model” of complex cells; Adelson and Bergen 1985). In this case the quadratic component can be expected to dominate the sum at high contrast, leading to a largely phase-invariant response, while the linear component will dominate the sum when at low contrast, leading to a more modulated response. Analogously, in the sum $a s + b s^2$, the linear term, $as$, dominates when $s << 1$ while the quadratic term, $bs^2$, dominates when $s >> 1$, where $s$ is a positive signal and $a$ and $b$ are constants.

In summary, we are not able to provide a definitive model to explain the contrast-dependent phase sensitivity that we observe, but it is clear that future cortical models must account for a range of contrast-dependent effects such as changes in RF size, changes in spatial frequency tuning, contrast adaptation, and changes in spatial phase sensitivity.

A very clear finding from our results is that complex cells that show increased spatial phase sensitivity at low contrast are overrepresented in supragranular layers (layers 1–3) or, in one cell population, also in granular layer 4 of the cortex. Sub-
granular layers (5–6) contain comparatively fewer such cells. Henry and Hawken (2013) and Cloherty and Ibbotson (2015) investigated the laminar distribution of contrast-dependent phase sensitivity in primate V1 and found such cells to be in similar proportions across layers. In contrast, given the large cell population analyzed in the present work, there is no doubt that there is a laminar bias in the cat. The significance of the cat result is increased by the fact that two techniques were used on at least some of the same cells (drifting and contrast-reversing gratings) and both reveal an overrepresentation in the supragranular layers. It is possible that there is a species difference between cat and monkey in terms of the connectivity between layers. However, it is also worth noting that the monkey studies were based on calculating F1-to-F0 modulation ratios, while here we used a new metric for the analysis of the data from both the drifting and contrast-reversing gratings, which relies on finding peak responses. It might be that this metric is more sensitive than the more commonly used Fourier analysis technique used to generate F1/F0.

**REFERENCES**


