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Research Article: New Research | Sensory and Motor Systems

Synaptic basis for contrast-dependent shifts in functional identity in mouse V1

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https://doi.org/10.1523/ENEURO.0480-18.2019

Received: 11 December 2018

Revised: 12 February 2019

Accepted: 27 February 2019

Published: 20 March 2019

M.Y., N.J.P., and M.R.I. designed research; M.Y. and V.C. performed research; M.Y., V.C., H.M., S.L.C., N.J.P., and M.R.I. analyzed data; M.Y., V.C., N.J.P., and M.R.I. wrote the paper.

Funding: Australian Research Council Centre of Excellence for Integrative Brain Function
CE140100007
;

Funding: National Health and Medical Research Council
GNT0525459
;

Funding: L.E.W. Carty Charitable Fund & Lions Foundation of Victoria
;

Funding: National Health Institutes
EY-020592
.

Conflict of Interest: The authors report no conflict of interest.

M.Y. and V.C. Equal contribution

N.J.P. and M.R.I. Equal contribution

This work was supported by the Australian Research Council Centre of Excellence for Integrative Brain Function (CE140100007), the National Health and Medical Research Council (GNT0525459), the L.E.W. Carty Charitable Fund & Lions Foundation of Victoria and the National Health Institutes (EY-020592).

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Cite as: eNeuro 2019; 10.1523/ENEURO.0480-18.2019

Manuscript Title: Synaptic basis for contrast-dependent shifts in functional identity in mouse V1

Abbreviated Title: Contrast-dependent phase sensitivity in primary visual cortex: an intracellular study

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Number of Figures: 8
Number of Tables: 0
Number of Multimedia: 0

Number of Words:

Abstract:	*190*
Significance Statement:	*119*
Introduction:	*650*
Discussion:	*751*

Acknowledgements and Funding Sources: This work was supported by the Australian Research Council Centre of Excellence for Integrative Brain Function (CE140100007), the National Health and Medical Research Council (GNT0525459), the L.E.W. Carty Charitable Fund & Lions Foundation of Victoria and the National Health Institutes (EY-020592).

Conflict of interest: The authors report no conflict of interest.

Abstract

A central transformation that occurs within mammalian visual cortex is the change from linear, polarity-sensitive responses to nonlinear, polarity-insensitive responses. These neurons are classically labelled as either simple or complex, respectively, on the basis of their response linearity (Skottun et al., 1991). While the difference between cell classes is clear when the stimulus strength is high, reducing stimulus strength diminishes the differences between the cell types and causes some complex cells to respond as simple cells (Crowder et al., 2007; van Kleef et al., 2010; Hietanen et al., 2013). To understand the synaptic basis for this shift in behaviour we used *in vivo* whole cell recordings while systematically shifting stimulus contrast. We find systematic shifts in the degree of complex cell responses in mouse primary visual cortex (V1) at the subthreshold level, demonstrating that synaptic inputs change in concert with the shifts in response linearity and that the change in response linearity is not simply due to the threshold nonlinearity. These shifts are consistent with a visual cortex model in which the recurrent amplification acts as a critical component in the generation of complex cell responses (Chance et al., 1999).

Significance statement

The discovery of simple and complex cells in the primary visual cortex (V1) has been fundamental to our understanding of visual processing. While both cell types are orientation selective, simple cells are spatial phase sensitive while complex cells are phase-invariant. Extracellular recordings have shown that the responses of complex cells become phase sensitive at lower stimulus contrasts, suggesting more flexibility in processing mechanisms than previously thought. The mechanism by which this flexibility arises is not understood. Using *in vivo* whole cell recordings, we demonstrated that the flexibility in phase sensitivity is also apparent in the subthreshold responses of mouse V1 cells, suggesting that the effect arises from active cortical recurrent network activity and not from passive spiking threshold mechanisms.

Introduction

The receptive fields (RFs) of cells in the primary visual cortex (V1) are classified as either simple or complex based on their spatial organisation (Hubel and Wiesel, 1962; Henry, 1977). Simple cell RFs have segregated subfields that respond to either brightness increments (ON) or decrements (OFF); complex cells do not have clearly segregated ON and OFF subfields (Hubel and Wiesel, 1962; Gilbert, 1977; Henry, 1977; Hammond and Ahmed, 1985; Spitzer and Hochstein, 1988; Mechler and Ringach, 2002; Priebe et al., 2004; Hietanen et al., 2013). The Hubel & Wiesel hierarchical model proposed that convergent synaptic inputs are responsible for these transformations in two stages (Hubel and Wiesel, 1962): thalamic relay cells, displaced along an oriented axis, converge on simple cells, generating orientation selectivity, then simple cells converge on complex cells to provide polarity invariance.

The distinction between simple and complex cells is related to neuronal laminar position and synaptic connectivity in some mammals (Ringach et al., 2002; Martinez et al., 2005; Williams and Shapley, 2007). Simple cells are found more often in cortical layers that receive thalamocortical connections, while complex cells are found in layers with dense recurrent cortical connectivity. The differences between simple and complex cell RFs may reflect a general process in which cortical circuits generalize selectivity by amplifying inputs. While cortical amplification has previously been hypothesized to increase selectivity (Benyishai et al., 1995; Douglas et al., 1995; Somers et al., 1995), it is also possible for it to generalize selectivity by integrating inputs with distinct RFs. We examined whether simple and complex cell responses in V1 exhibited signatures of this amplification.

One quantitative method to distinguish simple and complex cells depends on the relative modulation of responses to drifting sinusoidal gratings (Skottun et al., 1991). When stimulated with high-contrast drifting gratings, simple cell responses modulate as the grating moves across the distinct ON and OFF subfields. In contrast, complex cells respond to all phases of the drifting gratings. Studies have demonstrated that the ratio (F_1/F_0) of the modulated spiking component (F_1) to the unmodulated component (F_0) forms a bimodal distribution, suggesting two classes of V1 neurons (Maffei and Fiorenti, 1973; Movshon et al., 1978; De Valois et al., 1982; Skottun et al., 1991). While this difference between cell classes is clear when the stimulus strength is high, reducing stimulus strength diminishes the differences between the cell types. In particular, low contrast gratings evoke modulated responses in many complex cells (cat: Crowder et al., 2007; van Kleef et al., 2010; monkey: Henry and Hawken, 2013; Cloherty and Ibbotson, 2015; Meffin et al., 2015).

The mechanism underlying this change in spiking modulation ratio is not understood but there are two candidate models. The first model suggests that modulations in response to low contrast stimuli emerge due to the "iceberg" effect in which not all synaptic responses are converted into spikes (Carandini and Ferster, 2000; Mechler and Ringach, 2002; Priebe et al., 2004). In this model the subthreshold synaptic modulation ratio (V_1/V_0) should not depend on contrast. Alternatively, there may be a shift in the synaptic inputs to V1 neurons in which the V_1/V_0 ratio increases as the contrast decreases. A cortical model in which the amplification acts to integrate inputs with distinct spatial preferences predicts this specific change in synaptic input.

To distinguish these possibilities, we performed *in vivo* whole cell recordings in mouse V1. Both the V_1/V_0 and the F_1/F_0 ratios increased as contrast was reduced indicating that a change in synaptic drive is the more likely explanation for the altered modulation responses of complex cells. We have demonstrated that the circuitry leading to spatial phase invariant responses in visual cortex depends on the strength of visual drive. This observation is consistent with a scheme of complex cell generation in which the recurrent inputs in the visual cortex act as amplifiers, generating linear or nonlinear responses when input gain is low or high, respectively (Chance et al., 1999).

Materials and Methods

Electrophysiology

Recordings were made from anaesthetised C57BL/6 mice of both sexes aged five to twelve weeks. All 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 experimental procedures were approved by Animal Ethics Committees of the University of Melbourne, or by the Institutional Animal Care and Use Committee of the University of Texas at Austin. Mice were anesthetised with intraperitoneal injections of chloroprothixene (10mg/kg) followed by Urethane (1g/kg). Animals also received an injection of dexamethasone (2mg/kg) to reduce brain oedema. The level of anaesthesia was monitored using the electrocardiogram (ECG) and repeated toe-pinches throughout the experiment. Body temperature was monitored and maintained at 37°C using an auto-regulating heating blanket. A tracheotomy was performed to ensure a clear airway. A craniotomy approximately 1mm × 2.5 mm was performed over V1 in one hemisphere and the dura mater retracted.

Intracellular responses were obtained in mice via blind recordings with a whole-cell configuration *in vivo* as previously described (Ferster and Jagadeesh, 1992; Margrie et al., 2002; Priebe et al., 2004; Tan et al., 2011). Patch pipettes with tip resistances of 8–10 MOhm were pulled from borosilicate glass capillaries (1.2 mm outer diameter, 0.7 mm inner diameter; KG-33, King Precision Glass). A silver chloride coated silver wire was inserted into the pipette, which was filled with 135 mM K-gluconate, 4 mM NaCl, 0.5 mM EGTA, 2 mM MgATP, 10 mM phosphocreatine disodium, and 10 mM HEPES, pH adjusted to 7.3 with KOH (Sigma-Aldrich). A silver–silver chloride wire was

inserted as a reference electrode into muscles near the base of the skull. The craniotomy as well as the reference electrode was covered with 4% agarose in normal saline to keep the cortex moist and to reduce changes in the surrounding fluid and concomitant changes in associated junction potentials. An Axoclamp 2B patch-clamp amplifier was used in current clamp to record from neurons 150–600 μm below the surface of the cortex. The voltage was digitised and recorded with custom software (Labview, National Instruments), which also sent instructions to a separate stimulus-generation computer.

Visual Stimuli

Visual stimuli were generated using the Psychophysics toolbox for Matlab (The Mathworks Inc. Natick, MA, USA) and were presented on a calibrated CRT monitor (Sony GDM-F520, 100 Hz non-interlaced refresh rate, 1280x1024 pixels, 25 cd/m^2 mean luminance). The viewing distance for all recordings was 30 cm. For each recorded cell we measured its orientation, spatial frequency and temporal frequency preferences, as well as its RF location and size using drifting sinusoidal gratings. For example, to determine orientation preference, sinusoidal gratings were presented at eight different orientations (0, 22.5, 45, 67.5, 90, 112.5, 135, 157.5°). After 0.5 s presentation of each orientation, gratings moving in the opposite direction were presented and followed with 0.5 s of grey screen (at the mean luminance of the prior grating). The optimal tuning parameters were determined online and then applied to the experimental stimuli. The contrast of the grating was defined as: Michelson contrast = $[(Lum_{max} - Lum_{min})/Lum_{max} + Lum_{min})] \times 100$ where Lum_{max} and Lum_{min} are the maximum and minimum luminance of the grating.

Two types of experimental stimuli were used: drifting sinusoidal gratings and sinusoidally modulated contrast-reversing gratings. Stimuli were presented at the optimal temporal frequency (TF), spatial frequency (SF) and orientation of the recorded cell in a circular aperture the size of its excitatory RF. Drifting gratings with contrast levels ranging between 4 and 100% were presented in pseudorandom order interleaved with 1 s presentations of a blank (mean luminance) screen. Each grating was presented for 3 s with the first and last 0.5 s stationary, and drifting for the 2 s in between. Trials were repeated as many times as the stability of the recording would allow. Contrast-reversing gratings were presented at 8 different spatial phases (0, 45, 90, 135, 180, 225, 270, 315°). Depending on the recording stability, various combinations of contrast between 8 and 100% were tested. Each stimulus presentation consisted of a grating presented for 0.5 s with a steady contrast, 2 s presented with sinusoidally modulated contrast, and another 0.5 s with steady contrast.

Data analysis

The resting membrane potential (V_{rest}) of a patched cell, measured as the responses to a blank screen (0% contrast), ranged from -40 mV to -80 mV. To examine the subthreshold membrane potential modulation, spikes were removed from the raw records prior to analysis using a 5 ms median filter (Jagadeesh et al., 1997). The modulation ratios for membrane potential (V_1/V_0) and spiking rate (F_1/F_0) to drifting gratings were calculated as previously described in Priebe et al. (2004). For contrast-reversing gratings, the modulation ratios for membrane potential and spiking rate were calculated as V_2/V_1 and F_2/F_1, respectively. Cycle-averaged responses were measured by aligning each

response cycle, excluding the first cycle. The mean and standard error of the membrane potential and spiking rate were calculated at each time point of the cycle-averaged response. As in Priebe et al. 2004, the mean membrane potential (V_0) and spiking rate (F_0) are based on the differences between the responses during a stimulus and the spontaneous responses during a blank screen of the same time. Fourier coefficients at the fundamental frequency of the stimulus grating (V_1 for membrane potential, F_1 for spiking rate) and at twice the stimulus input (V_2 for membrane potential, F_2 for spiking rate) for each cycle-averaged response were extracted using the FFT function in Matlab (The Mathworks Inc. Natick, MA, USA). A perfect half-wave rectified spiking rate response is expected to have an F_1/F_0 ratio of 1.57. We did find two cells with F_1/F_0 ratios above 1.57 at high stimulus contrasts, but this was due to low F_0 values created from subtracting high spontaneous spiking rate from evoked spiking rate. All cells showed significant increases in mean spiking rate (F_0) relative to the spontaneous spiking rate ($p < 0.05$, one-sided t-tests). One cell with a modulation ratio (F_1/F_0) of 2.67 had a relatively high spontaneous spiking rate (6.9 spks/s) compared to evoked spiking rate (9.6 spks/s). The other cell with F_1/F0 of 1.98 showed a relatively high spontaneous spiking rate (1.2 spks/s) compared to evoked spiking rate (6.5 spks/s). Both cells showed significant increases in evoked F_1 amplitude with a clear response to the drifting grating stimulus ($p = 0.0002$ and $p < 0.0001$, one-sided t-tests). Error bars were generated by projecting the cycle-by-cycle estimate of modulation amplitude and phase onto the mean phase and amplitude vector in complex space.

Model

Each neuron in the network model receives feedforward and recurrent input and is based on the rate model developed by Chance, Nelson and Abbott (1999). The activity of neuron *i*, is modelled using a simple rate model equation that includes a threshold nonlinearity:

$$\tau_v \frac{dv_i}{dt} = -v_i + I_i^{ff} + I_i^{rec} \quad (1)$$

$$r_i = \lfloor v_i - v_{thresh} \rfloor_+ \quad (2)$$

where I_i^{ff} and I_i^{rec} represent the feedforward and recurrent inputs. We use a time constant, τ_r of 20 ms and a positive voltage threshold (v_{thresh}). The feedforward input is equal to a half-wave rectified sinuosoidal modulation, where each network neuron has a random preferred spatial phase (ϕ_i).

$$I_i^{ff} = \sin\left(\frac{2*\pi t}{500} - \phi_i\right) \quad (3)$$

The recurrent input to model neuron *i* is given by:

$$I_i^{rec} = \frac{(1 - \varepsilon_i)}{N} r_i \quad (4)$$

The degree of recurrent and feedforward input is set by the value of ε_i, which was randomly set between 0 and 1, with zero reflecting all recurrent input and one reflecting all feedforward input.

Results

We first explored a model of the transformation between simple and complex cells to guide our experiments based on the architecture from Chance et al. (1999). They used a rate model to demonstrate that the degree of simple cell and complex cell behaviour is related to the amount of recurrent circuitry in the network. We implemented their model with two changes. First, each neuron received a random degree of feedforward input ($\sqrt{K_i}$) so that we would observe both simple and complex cells within the same network. Second, we included a non-zero threshold to model the threshold nonlinearity between the input and output. Both simple and complex cells emerge from this network model, as seen in the modulation ratio to a drifting grating (Fig. 1A). Simple cells respond at one phase of the stimulus and have a modulation ratio that is greater than 1 (Fig. 1A, top row). Network complex cells exhibit a response that varies little with the drifting grating and are characterized by a modulation ratio less than 1 (Fig. 1A, bottom row). Importantly in this network simulation we can view neurons that exhibit combinations of linear and nonlinear components (Fig 1A, middle row) and therefore have a modulation ratio between 0.5 and 1.

As the emergence of complex cells in this model depends on the degree of recurrent amplification, we hypothesized that reducing the input strength would impact the modulation ratios of network neurons, and thus the degree of generalization across spatial phase. Indeed, we find that reducing input strength, or visual contrast, leads to systematic increases in the modulation ratios of network neurons (Fig. 1B). The modulation ratios of model neurons shift to higher values as the input strength is reduced, even switching neurons that

would be classified as complex for high contrast to being simple at low contrast (Fig. 1A, middle row). Contrast-dependent changes in the modulation ratio could therefore reflect the amplification structure of the model visual cortex. There are two components that contribute to the increase in modulation ratio with contrast. First, the observed increase in the network modulation ratio with contrast depends on the voltage threshold (V_{thresh}). If the voltage threshold is set to 0 then no change in modulation ratio occurs with changes in contrast (data not shown). Second, there are systematic increases in the synaptic modulation ratio (Fig. 1C) as contrast is reduced. This simple model demonstrates how the cortical circuitry, acting as an amplifier, could generate spatially invariant responses and demonstrates that the degree of the spatial invariance depends on the input strength.

There are other possible models that could explain the shift in modulation ratio due to changes in contrast. One alternative possibility is an "iceberg" effect where not all synaptic responses are converted into spiking activities (Carandini and Ferster, 2000; Mechler and Ringach, 2002; Priebe et al., 2004). For a high contrast stimulus, the synaptic input is sufficient to evoke spiking responses at all phases (Hietanen et al. 2013), whereas for a low contrast stimulus, the synaptic input falls below threshold and is only sufficient to evoke spiking responses for a subset of phases (Fig. 2A). In this model, the modulation ratio of the synaptic input (V_1/V_0: the modulation ratio of the membrane potential) does not vary (Fig. 2B); instead the change in the spiking modulation ratio is due to the threshold nonlinearity. This explanation for the observed changes in the spike modulation ratio with contrast proposes that the underlying

membrane potential modulation ratio is fixed and the changes observed at the level of spiking emerge from the threshold nonlinearity.

To examine whether signatures of these models exist in V1 neuron responses we measured the degree to which the modulation ratio of the synaptic input varies with contrast. Previous experimental reports have demonstrated that the spiking modulation ratio of V1 neurons is contrast dependent, which matches the pattern shown in the model, i.e. responses become more simple-like as contrast declines. However, these records do not differentiate between synaptic changes from the network and changes that may exclusively emerge from threshold nonlinearity. To determine whether the change in the spiking modulation ratio is due to threshold or synaptic mechanisms, we recorded intracellularly from V1, giving us access to both the underlying membrane potential as well as the spiking rate in response to gratings. We recorded from 20 cells with drifting gratings and 21 cells with contrast reversing gratings in 20 urethane-anaesthetised mice.

Responses to drifting gratings

Based on responses to drifting sinusoidal gratings, mouse V1 neurons show the same separation into simple and complex cells as cats and primates (Niell and Stryker, 2008). We classified cells as simple by the large modulation of spiking rate ($F_1/F_0 > 1$) to a drifting grating stimulus. The underlying membrane potential of these neurons also exhibited large modulations when stimulated at the preferred orientation, spatial frequency and temporal frequency (Fig. 3, 100% contrast). Membrane potential fluctuations were separated from spiking rate by identifying the spike times and removing them from the membrane

potential traces using a median filter (see Methods). Both the raw response and the trial-averaged membrane potential for the simple cell in Figure 3 are highly modulated at the input frequency and phase-locked to the sinusoidal grating stimulus (Fig. 3, bottom).

Previous work has demonstrated that the classification of simple cells does not vary with contrast in the cat and primate (cat: Crowder et al., 2007; van Kleef et al., 2010; monkey: Henry and Hawken, 2013; Cloherty and Ibbotson, 2015; Meffin et al., 2015). We first examined whether this is also true in mouse V1 by measuring the changes in the modulation ratios for spiking rate (F_1/F_0) and membrane potential (V_1/V_0) of individual simple cells with contrast (Fig. 4A). For simple cells, V_1/V_0 did vary with contrast but the F_1/F_0 was consistently higher than unity, indicating that simple cell classification does not depend on contrast (Fig. 4A). Across our sample population we found that the subthreshold modulation ratio (V_1/V_0) of simple cells often increased with decreasing contrast but this change was not statistically significant ($n = 13$, $p > 0.05$, one-sided t-test; red symbols in Fig. 5A). This result is consistent with results from an earlier study, in which simple cells in cat V1 showed increased V_0 and V_1 as contrasts decreased (Carandini and Ferster, 1997). Despite those subthreshold changes, however, the F_1/F_0 ratio was consistently above unity for simple cells at low and high contrasts (Fig. 5B). Therefore, the simple cell population remains highly phase sensitive at both the membrane potential and spiking output levels for all contrasts.

We next examined how contrast alters the modulation ratio of complex cells in mouse V1. As found in other mammals, complex cells modulate more at low contrasts than high contrasts (cat: Crowder et al., 2007; van Kleef et al.,

2010; monkey: Henry and Hawken, 2013; Cloherty and Ibbotson, 2015; Meffin et al., 2015). We found a range of contrast-dependent shifts in the modulation ratios, which demonstrate that synaptic mechanisms are involved in this process. For some complex cells modulations in response amplitude are clearly evoked across all contrasts at the level of both the membrane potential and spiking rate (Fig. 4B). Measures of the modulation ratios of the membrane potentials systematically increase as contrast decreases. This shift is matched by a commensurate increase in the F_1/F_0 ratios. For both spiking rate and membrane potential responses, the mean responses (F_0 & V_0) dominate the modulation amplitudes (F_1 & V_1) at high contrasts (Fig. 4B). As the contrast decreases, however, the differences between these two parameters declines and results in increased modulation ratios. This trend is especially prominent in the membrane potential responses in which V_1 remains largely unchanged compared to V_0. When considering the complex cell and the simple cell examples together, it is noticeable that similar membrane potential characteristics in the two example cells (Fig. 4A & B) are observed at higher contrasts (32% and 64%). Both cells show substantial modulations of the fundamental frequencies of the input (V_1) that are well above the resting membrane potential, which result in the V_0 component being larger than the V_1 component. However, the spiking responses show different response characteristics in the two cells: cell A has a larger F_1 component at high contrasts whereas cell B has a larger F_0 component (Fig. 4A & 4B). As a result, cell A has an $F_1/F_0 > 1$ and is therefore classified as a simple cell, whereas Cell B is classified as a complex cell because it has an $F_1/F_0 < 1$. These observations suggest that the dichotomy between simple and complex cells based on spiking modulation ratios with high stimulus strengths does not

directly translate to corresponding distinctions in the membrane potential responses. The differences in the F_1/F_0 ratios in the two cells are likely the result of non-linear threshold transformations from the membrane potentials to the spiking outputs (Priebe et al., 2004).

At the population level, changes in the F_1/F_0 (spiking rate) and V_1/V_0 (membrane potential) ratios of complex cells at high and low contrasts have characteristics similar to the responses of the synaptic model. The scatter plots of both F_1/F_0 and V_1/V_0 show significant increases at low contrast compared to high contrast (Fig. 5). The distribution of V_1/V_0 ratios presented as a histogram reveal a significant shift towards higher values at low contrasts compared to high contrasts ($n = 20$, $p = 0.008$, one-sided t-test; blue symbols in Fig. 5A). The population spiking responses also show significant increases in F_1/F_0 ratios at low contrasts ($n = 20$, $p = 0.02$, one-sided t-test, Fig. 5B).

Responses to contrast reversing gratings

An alternative method to quantify the nonlinearities of cortical neurons is to examine the modulated responses to contrast-reversing gratings (Hawken and Parker, 1987). An ideal simple cell should modulate at the temporal frequency of the contrast reversal (F_1), and the timing of its response should depend on the spatial phase of the grating (Fig. 6, left). An ideal complex cell should modulate at twice the temporal frequency of the contrast reversal (F_2), and the timing of its response should not depend on the spatial phase of the grating (Fig. 6, right). One can then distinguish simple and complex cells by considering the first and second Fourier components in the complex plane (Fig. 6B). Simple cells should have large F_1 components that lie along an axis in the complex plane. For the

example simple cell, that axis is along the abscissa. In contrast, the example complex cell has small F_1 components, but a large F_2 component for which the response does not change with the stimulus phase. To extract a metric that describes the relative F_1 and F_2 modulations of the responses, we computed the amplitude of the projection of the F_1 values onto their principle axis in the complex plane, and compared that to the vector average F_2 value in the complex plane. Doing so enforces the expectation that the timing of the F_2 component should be invariant to spatial phase. The resulting contrast reversing modulation index (F_2/F_1) is large for complex cells and small for simple cells.

To quantify how much V1 neurons shift to more simple-like behaviour as contrast is lowered, we presented contrast-reversing gratings at eight different spatial phases and extracted the phase and amplitude of the Fourier components at the temporal frequency of the reversing gratings and at twice the temporal frequency of the reversing gratings (Fig. 7). These measurements were made both for the spiking rate of the neurons and their underlying membrane potentials.

As with drifting gratings we found that reductions in contrast caused systematic changes in membrane potential modulations that reflected a shift toward more simple-like behaviour in complex cells. At high contrast these complex cells are characterized by frequency doubled responses in both membrane potential and spiking rate (Fig. 7A). When contrast was lowered, however, the amplitude of the frequency doubled responses declined relative to the modulation at the temporal frequency of the reversing grating. Note that not only do modulations emerge at low contrasts, but the timing of the modulations

406 systematically shifts with the spatial phases of the gratings, as predicted by an
407 ideal simple cell (Fig. 6).

408 As shown in the membrane potential and spiking rate traces, the
409 projected F_2 and F_1 modulations vary across stimulus phase (Fig. 7B). To
410 quantify the changes outlined above for each cell, we estimated individual F_1 &
411 F_2 (spikes) and V_1 & V_2 (membrane potentials) values across all spatial phases
412 for each stimulus contrast tested. For F_2 and V_2, we simply averaged across all
413 stimulus spatial phases since these values were spatial phase-invariant (red lines
414 in Fig. 6C and Fig. 7B). However, averaging across all stimulus spatial phases
415 does not work for F_1 and V_1 because they are spatial phase dependent (blue lines
416 in Fig. 6C and Fig. 7B). At high contrasts the amplitudes of the F_2 and V_2
417 modulations do not modulate with spatial phase, while the F_1 and V_1 components
418 clearly modulate. When contrast is lowered, the F_2 and V_2 modulation
419 amplitudes decline more rapidly relative to the F_1 and V_1 components,
420 respectively. These changes cause an overall decline in the membrane potential
421 modulation ratio (V_2/V_1) from 0.7 at high contrast to 0.12 at low contrast (Fig.
422 8A, arrow). As small modulation ratios are associated with simple cells and
423 larger ones with complex cells, this is an example in which contrast shifts the
424 behaviour of the neuron toward more simple-like responses.

425 To quantify how contrast altered the behaviour of our complex cell
426 population we estimated V_2/V_1 and F_2/F_1 modulation indices across our sample
427 population (membrane potential: n = 21, spikes: n = 12; Fig. 8). We found that
428 the membrane potential modulation ratio systematically declined with contrast,
429 changing from a mean value of 0.83 to 0.57 ($P = 0.016$, one-sided t-test). There
430 was a similar, but more modest, change in the modulation ratios obtained from

spiking rate across our sample population (P = 0.021, one-sided t-test). Therefore, as with drifting gratings, neurons in mouse visual cortex shift toward more simple-like responses as contrast is lowered.

Discussion

Contrast-dependent phase sensitivity has been documented in a subpopulation of neurons in the primary visual cortex of cat (Crowder et al., 2007; van Kleef et al., 2010; Hietanen et al., 2013) and primate (Henry and Hawken, 2013; Cloherty and Ibbotson, 2015). The current study demonstrates that contrast-dependent phase sensitivity is also present in the primary visual cortex of mouse. Cortical visual processing in mice has been studied extensively in the past decade (Niell and Stryker, 2008; Huberman and Niell, 2011; Tan et al., 2011). The abundant opportunities for genetic manipulation have made mouse visual cortex a useful model in addition to carnivores and primates for studying RF properties (e.g. Wang et al., 2006; Liu et al., 2009; Zariwala et al., 2011). All previous literature showing contrast-dependent changes in response linearity in cats and monkeys has been quantified using modulation ratios calculated from extracellular responses to drifting sinusoidal gratings (Crowder et al., 2007; van Kleef et al., 2010; Henry and Hawken, 2013; Hietanen et al., 2013; Cloherty and Ibbotson, 2015) or contrast-reversing gratings (Meffin et al., 2015).

We used whole cell recordings to shed light on how complex cells emerge in V1. A simple model to describe this process is that recurrent cortical connectivity between neurons with distinct spatial selectivity generates the spatial-invariant responses that characterize complex cells. Two models have been proposed to describe this shift, one from Hubel and Wiesel in which simple cells receive inputs from the dorsal lateral geniculate nucleus (dLGN) in the thalamus and converge onto complex cells in one step to generate spatial invariance (Hubel and Wiesel, 1962). Alternatively the generation of spatial invariance may require many steps, which reflect an increase in the proportion

460 of cortical circuitry that neurons receive (Chance et al., 1999). One way to
461 distinguish these possibilities is to observe how input strength alters the
462 emergence of complex cells. Mouse LGN neurons show mostly linear contrast
463 sensitivity, the responses of individual LGN cells increase with increasing
464 contrasts (Grubb and Thompson, 2003; Tang et al., 2016). Lien and Scanziani
465 (2013) have shown that recurrent cortical excitation to simple cells in mouse V1
466 are phase-sensitive and matches their LGN inputs. However, it is unclear if this is
467 the case for complex cells. We demonstrate that as contrast declines both
468 membrane potential and spiking modulation ratios increase, as expected from
469 the recurrent model proposed by Chance et al. (1999).

470 An alternative explanation for the shift in modulation ratio at low contrast
471 is the variability of contrast response curves across simple cells. Neurons within
472 V1 vary in the contrast at which they saturate, such that for some neurons the
473 changes in contrast may yield large changes in response amplitude whereas for
474 others they may evoke little effect (Van den Bergh et al. 2010). A simple model
475 that includes the variance in the contrast response curves of simple cells which
476 converge onto a complex could only account for input modulation ratio shifts of
477 less than 0.1, relative to our measures of modulation ratio shifts of more than
478 0.45 (data not shown).

479 We analysed the responses to drifting gratings and found that there is a
480 shift in the input modulation ratio (V_1/V_0) with contrast consistent with a
481 synaptic model (mean V_1/V_0 high contrast: 0.62, low contrast: 1.09). While the
482 threshold nonlinearity may play a role in altering the phase sensitivity of
483 neurons (Priebe et al., 2004), there is a clear synaptic component to the shift in
484 phase sensitivity. Also, as expected, when stimulated with high-contrast

485 reversing gratings these cells exhibited various degrees of frequency-doubled
486 responses (Meffin et al., 2015). However, at low contrasts, the same cells showed
487 more modulated, phase sensitive responses to drifting gratings and a tendency
488 to respond to selected spatial phases during stimulation with contrast-reversing
489 gratings.

490 In summary, for some years now it has been noted that complex cells
491 show increased modulatory responses at low contrasts, suggesting that they are
492 more phase sensitive at low contrasts (Crowder et al., 2007; van Kleef et al.,
493 2010; Henry and Hawken, 2013; Cloherty and Ibbotson, 2015; Meffin et al.,
494 2015). We demonstrate that this is not simply a manifestation of the 'iceberg'
495 phenomenon (Carandini and Ferster, 2000; Mechler and Ringach, 2002; Priebe
496 et al., 2004), but instead a systematic shift in the inputs that cortical neurons
497 receive. This network level change in input modulation with contrast is
498 consistent with a model for the generation of invariant responses in which
499 complex cells emerge steadily through the cortical network through increases in
500 the degree of recurrent inputs that they receive.

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