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

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28 Abstract

29 A central transformation that occurs within mammalian visual cortex is the change 30 from linear, polarity-sensitive responses to nonlinear, polarity-insensitive responses. 31 These neurons are classically labelled as either simple or complex, respectively, 32 on the basis of their response linearity (Skottun et al., 1991). While the 33 difference between cell classes is clear when the stimulus strength is high, 34 reducing stimulus strength diminishes the differences between the cell types and 35 causes some complex cells to respond as simple cells (Crowder et al., 2007; van 36 Kleef et al., 2010; Hietanen et al., 2013). To understand the synaptic basis for 37 this shift in behaviour we used *in vivo* whole cell recordings while systematically 38 shifting stimulus contrast. We find systematic shifts in the degree of complex cell 39 responses in mouse primary visual cortex (V1) at the subthreshold level, 40 demonstrating that synaptic inputs change in concert with the shifts in response 41 linearity and that the change in response linearity is not simply due to the 42 threshold nonlinearity. These shifts are consistent with a visual cortex model in 43 which the recurrent amplification acts as a critical component in the generation 44 of complex cell responses (Chance et al., 1999).

46 Significance statement

47 The discovery of simple and complex cells in the primary visual cortex (V1) has 48 been fundamental to our understanding of visual processing. While both cell 49 types are orientation selective, simple cells are spatial phase sensitive while 50 complex cells are phase-invariant. Extracellular recordings have shown that the 51 responses of complex cells become phase sensitive at lower stimulus contrasts, 52 suggesting more flexibility in processing mechanisms than previously thought. 53 The mechanism by which this flexibility arises is not understood. Using *in vivo* 54 whole cell recordings, we demonstrated that the flexibility in phase sensitivity is 55 also apparent in the subthreshold responses of mouse V1 cells, suggesting that 56 the effect arises from active cortical recurrent network activity and not from 57 passive spiking threshold mechanisms.

58 Introduction

59 The receptive fields (RFs) of cells in the primary visual cortex (V1) are 60 classified as either simple or complex based on their spatial organisation (Hubel and Wiesel, 1962; Henry, 1977). Simple cell RFs have segregated subfields that 61 62 respond to either brightness increments (ON) or decrements (OFF); complex 63 cells do not have clearly segregated ON and OFF subfields (Hubel and Wiesel, 1962; Gilbert, 1977; Henry, 1977; Hammond and Ahmed, 1985; Spitzer and 64 65 Hochstein, 1988; Mechler and Ringach, 2002; Priebe et al., 2004; Hietanen et al., 2013). The Hubel & Wiesel hierarchical model proposed that convergent 66 67 synaptic inputs are responsible for these transformations in two stages (Hubel 68 and Wiesel, 1962): thalamic relay cells, displaced along an oriented axis, 69 converge on simple cells, generating orientation selectivity, then simple cells 70 converge on complex cells to provide polarity invariance.

71 The distinction between simple and complex cells is related to neuronal 72 laminar position and synaptic connectivity in some mammals (Ringach et al., 73 2002; Martinez et al., 2005; Williams and Shapley, 2007). Simple cells are found 74 more often in cortical layers that receive thalamocortical connections, while 75 complex cells are found in layers with dense recurrent cortical connectivity. The 76 differences between simple and complex cell RFs may reflect a general process in 77 which cortical circuits generalize selectivity by amplifying inputs. While cortical 78 amplification has previously been hypothesized to increase selectivity 79 (Benyishai et al., 1995; Douglas et al., 1995; Somers et al., 1995), it is also 80 possible for it to generalize selectivity by integrating inputs with distinct RFs. We 81 examined whether simple and complex cell responses in V1 exhibited signatures 82 of this amplification.

83 One quantitative method to distinguish simple and complex cells depends 84 on the relative modulation of responses to drifting sinusoidal gratings (Skottun 85 et al., 1991). When stimulated with high-contrast drifting gratings, simple cell 86 responses modulate as the grating moves across the distinct ON and OFF 87 subfields. In contrast, complex cells respond to all phases of the drifting gratings. 88 Studies have demonstrated that the ratio (F_1/F_0) of the modulated spiking 89 component (F_1) to the unmodulated component (F_0) forms a bimodal 90 distribution, suggesting two classes of V1 neurons (Maffei and Fiorenti, 1973; 91 Movshon et al., 1978; De Valois et al., 1982; Skottun et al., 1991). While this 92 difference between cell classes is clear when the stimulus strength is high, 93 reducing stimulus strength diminishes the differences between the cell types. In 94 particular, low contrast gratings evoke modulated responses in many complex 95 cells (cat: Crowder et al., 2007; van Kleef et al., 2010; monkey: Henry and 96 Hawken, 2013; Cloherty and Ibbotson, 2015; Meffin et al., 2015).

97 The mechanism underlying this change in spiking modulation ratio is not 98 understood but there are two candidate models. The first model suggests that 99 modulations in response to low contrast stimuli emerge due to the "iceberg" 100 effect in which not all synaptic responses are converted into spikes (Carandini 101 and Ferster, 2000; Mechler and Ringach, 2002; Priebe et al., 2004). In this model 102 the subthreshold synaptic modulation ratio (V_1/V_0) should not depend on 103 contrast. Alternatively, there may be a shift in the synaptic inputs to V1 neurons 104 in which the V_1/V_0 ratio increases as the contrast decreases. A cortical model in 105 which the amplification acts to integrate inputs with distinct spatial preferences 106 predicts this specific change in synaptic input.

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

117 Materials and Methods

118 Electrophysiology

119 Recordings were made from anaesthetised C57BL/6 mice of both sexes 120 aged five to twelve weeks. All experiments were performed according to the 121 National Health and Medical Research Council's Australian Code of Practice for the 122 Care and Use of Animals for Scientific Purposes. All experimental procedures were 123 approved by Animal Ethics Committees of the University of Melbourne, or by the 124 Institutional Animal Care and Use Committee of the University of Texas at Austin. 125 Mice were anesthetised with intraperitoneal injections of chloroprothixene 126 (10mg/kg) followed by Urethane (1g/kg). Animals also received an injection of 127 dexamethasone (2mg/kg) to reduce brain oedema. The level of anaesthesia was 128 monitored using the electrocardiogram (ECG) and repeated toe-pinches 129 throughout the experiment. Body temperature was monitored and maintained at 130 37°C using an auto-regulating heating blanket. A tracheotomy was performed to 131 ensure a clear airway. A craniotomy approximately 1mm × 2.5 mm was 132 performed over V1 in one hemisphere and the dura mater retracted.

133 Intracellular responses were obtained in mice via blind recordings with a 134 whole-cell configuration in vivo as previously described (Ferster and Jagadeesh, 135 1992; Margrie et al., 2002; Priebe et al., 2004; Tan et al., 2011). Patch pipettes 136 with tip resistances of 8-10 MOhm were pulled from borosilicate glass 137 capillaries (1.2 mm outer diameter, 0.7 mm inner diameter; KG-33, King 138 Precision Glass). A silver chloride coated silver wire was inserted into the 139 pipette, which was filled with 135 mM K-gluconate, 4 mM NaCl, 0.5 mM EGTA, 140 2 mM MgATP, 10 mM phosphocreatine disodium, and 10 mM HEPES, pH 141 adjusted to 7.3 with KOH (Sigma-Aldrich). A silver-silver chloride wire was 142 inserted as a reference electrode into muscles near the base of the skull. The 143 craniotomy as well as the reference electrode was covered with 4% agarose in 144 normal saline to keep the cortex moist and to reduce changes in the surrounding 145 fluid and concomitant changes in associated junction potentials. An Axoclamp 2B 146 patch-clamp amplifier was used in current clamp to record from neurons 150-147 600 µm below the surface of the cortex. The voltage was digitised and recorded 148 with custom software (Labview, National Instruments), which also sent 149 instructions to a separate stimulus-generation computer.

150

151 Visual Stimuli

152 Visual stimuli were generated using the Psychophysics toolbox for Matlab 153 (The Mathworks Inc. Natick, MA, USA) and were presented on a calibrated CRT 154 monitor (Sony GDM-F520, 100 Hz non-interlaced refresh rate, 1280x1024 pixels, 155 25 cd/m^2 mean luminance). The viewing distance for all recordings was 30 cm. 156 For each recorded cell we measured its orientation, spatial frequency and 157 temporal frequency preferences, as well as its RF location and size using drifting 158 sinusoidal gratings. For example, to determine orientation preference, sinusoidal 159 gratings were presented at eight different orientations (0, 22.5, 45, 67.5, 90, 160 112.5, 135, 157.5°). After 0.5 s presentation of each orientation, gratings moving 161 in the opposite direction were presented and followed with 0.5 s of grey screen 162 (at the mean luminance of the prior grating). The optimal tuning parameters 163 were determined online and then applied to the experimental stimuli. The 164 contrast of the grating was defined as: Michelson contrast = $[(Lum_{max} -$ 165 Lum_{min} / Lum_{max} + Lum_{min})] × 100 where Lum_{max} and Lum_{min} are the maximum 166 and minimum luminance of the grating.

167 Two types of experimental stimuli were used: drifting sinusoidal gratings 168 and sinusoidally modulated contrast-reversing gratings. Stimuli were presented 169 at the optimal temporal frequency (TF), spatial frequency (SF) and orientation of 170 the recorded cell in a circular aperture the size of its excitatory RF. Drifting 171 gratings with contrast levels ranging between 4 and 100% were presented in 172 pseudorandom order interleaved with 1s presentations of a blank (mean 173 luminance) screen. Each grating was presented for 3 s with the first and last 0.5 s 174 stationary, and drifting for the 2 s in between. Trials were repeated as many 175 times as the stability of the recording would allow. Contrast-reversing gratings 176 were presented at 8 different spatial phases (0, 45, 90, 135, 180, 225, 270, 315°). 177 Depending on the recording stability, various combinations of contrast between 178 8 and 100% were tested. Each stimulus presentation consisted of a grating 179 presented for 0.5 s with a steady contrast, 2 s presented with sinusoidally 180 modulated contrast, and another 0.5 s with steady contrast.

181

182 Data analysis

183 The resting membrane potential (V_{rest}) of a patched cell, measured as the 184 responses to a blank screen (0% contrast), ranged from -40 mV to -80 mV. To 185 examine the subthreshold membrane potential modulation, spikes were 186 removed from the raw records prior to analysis using a 5 ms median filter 187 (Jagadeesh et al., 1997). The modulation ratios for membrane potential (V_1/V_0) 188 and spiking rate (F_1/F_0) to drifting gratings were calculated as previously 189 described in Priebe et al. (2004). For contrast-reversing gratings, the modulation 190 ratios for membrane potential and spiking rate were calculated as V₂/V₁ and 191 F₂/F₁, respectively. Cycle-averaged responses were measured by aligning each

192	response cycle, excluding the first cycle. The mean and standard error of the
193	membrane potential and spiking rate were calculated at each time point of the
194	cycle-averaged response. As in Priebe et al. 2004, the mean membrane potential
195	(V ₀) and spiking rate (F ₀) are based on the differences between the responses
196	during a stimulus and the spontaneous responses during a blank screen of the
197	same time. Fourier coefficients at the fundamental frequency of the stimulus
198	grating (V $_1$ for membrane potential, F_1 for spiking rate) and at twice the stimulus
199	input (V_2 for membrane potential, F_2 for spiking rate) for each cycle-averaged
200	response were extracted using the FFT function in Matlab (The Mathworks Inc.
201	Natick, MA, USA). A perfect half-wave rectified spiking rate response is expected
202	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
203	at high stimulus contrasts, but this was due to low $F_{0}\xspace$ values created from
204	subtracting high spontaneous spiking rate from evoked spiking rate. All cells
205	showed significant increases in mean spiking rate (F_0) relative to the
206	spontaneous spiking rate (p < 0.05, one-sided t-tests). One cell with a
207	modulation ratio (F_1/F_0) of 2.67 had a relatively high spontaneous spiking rate
208	(6.9 spks/s) compared to evoked spiking rate (9.6 spks/s). The other cell with
209	$F_1/F0$ of 1.98 showed a relatively high spontaneous spiking rate (1.2 spks/s)
210	compared to evoked spiking rate (6.5 spks/s). Both cells showed significant
211	increases in evoked F_1 amplitude with a clear response to the drifting grating
212	stimulus (p = 0.0002 and p < 0.0001, one-sided t-tests). Error bars were
213	generated by projecting the cycle-by-cycle estimate of modulation amplitude and
214	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:

221
$$\Box_{v} \frac{dv_{i}}{dt} \Box I_{i}^{ff} \Box I_{i}^{rec} \Box v_{i}$$
(1)

222
$$r_i \Box \lfloor v_i \Box v_{thresh} \rfloor_{\Box}$$
(2)

223 where I_i^{ff} and I_i^{rec} represent the feedforward and recurrent inputs. We use a

time constant, \Box_r of 20 ms and a positive voltage threshold (v_{thresh}). The

225 feedforward input is equal to a half-wave rectified sinuosoidal modulation,

where each network neuron has a random preferred spatial phase (\Box_i).

227
$$I_{\underline{2}}^{ff} \square \square \sin(\frac{2^* \square t}{500} \square \square_i)$$
(3)

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

229
$$I_{\overrightarrow{[r]}}^{rec} \Box \frac{[1 \Box \Box_{i}]}{N} \Box r_{i}$$
(4)

230 The degree of recurrent and feedforward input is set by the value of \square_i , which

231 was randomly set between 0 and 1, with zero reflecting all recurrent input and

232 one reflecting all feedforward input.

234 **Results**

235 We first explored a model of the transformation between simple and 236 complex cells to guide our experiments based on the architecture from Chance et 237 al. (1999). They used a rate model to demonstrate that the degree of simple cell 238 and complex cell behaviour is related to the amount of recurrent circuitry in the 239 network. We implemented their model with two changes. First, each neuron 240 received a random degree of feedforward input (\Box_i) so that we would observe 241 both simple and complex cells within the same network. Second, we included a 242 non-zero threshold to model the threshold nonlinearity between the input and 243 output. Both simple and complex cells emerge from this network model, as seen 244 in the modulation ratio to a drifting grating (Fig. 1A). Simple cells respond at one 245 phase of the stimulus and have a modulation ratio that is greater than 1 (Fig. 1A, 246 top row). Network complex cells exhibit a response that varies little with the 247 drifting grating and are characterized by a modulation ratio less than 1 (Fig. 1A, 248 bottom row). Importantly in this network simulation we can view neurons that 249 exhibit combinations of linear and nonlinear components (Fig 1A, middle row) 250 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

258 would be classified as complex for high contrast to being simple at low contrast 259 (Fig. 1A, middle row). Contrast-dependent changes in the modulation ratio could 260 therefore reflect the amplification structure of the model visual cortex. There are 261 two components that contribute to the increase in modulation ratio with 262 contrast. First, the observed increase in the network modulation ratio with 263 contrast depends on the voltage threshold (V_{thresh}). If the voltage threshold is set 264 to 0 then no change in modulation ratio occurs with changes in contrast (data 265 not shown). Second, there are systematic increases in the synaptic modulation 266 ratio (Fig. 1C) as contrast is reduced. This simple model demonstrates how the 267 cortical circuitry, acting as an amplifier, could generate spatially invariant 268 responses and demonstrates that the degree of the spatial invariance depends on 269 the input strength.

270 There are other possible models that could explain the shift in modulation 271 ratio due to changes in contrast. One alternative possibility is an "iceberg" effect 272 where not all synaptic responses are converted into spiking activities (Carandini 273 and Ferster, 2000; Mechler and Ringach, 2002; Priebe et al., 2004). For a high 274 contrast stimulus, the synaptic input is sufficient to evoke spiking responses at 275 all phases (Hietanen et al. 2013), whereas for a low contrast stimulus, the 276 synaptic input falls below threshold and is only sufficient to evoke spiking 277 responses for a subset of phases (Fig. 2A). In this model, the modulation ratio 278 of the synaptic input (V_1/V_0) : the modulation ratio of the membrane 279 potential) does not vary (Fig. 2B); instead the change in the spiking modulation 280 ratio is due to the threshold nonlinearity. This explanation for the observed 281 changes in the spike modulation ratio with contrast proposes that the underlying 282 membrane potential modulation ratio is fixed and the changes observed at the283 level of spiking emerge from the threshold nonlinearity.

284 To examine whether signatures of these models exist in V1 neuron 285 responses we measured the degree to which the modulation ratio of the synaptic 286 input varies with contrast. Previous experimental reports have demonstrated 287 that the spiking modulation ratio of V1 neurons is contrast dependent, which 288 matches the pattern shown in the model, i.e. responses become more simple-like 289 as contrast declines. However, these records do not differentiate between 290 synaptic changes from the network and changes that may exclusively emerge 291 from threshold nonlinearity. To determine whether the change in the spiking 292 modulation ratio is due to threshold or synaptic mechanisms, we recorded 293 intracellularly from V1, giving us access to both the underlying membrane 294 potential as well as the spiking rate in response to gratings. We recorded from 295 20 cells with drifting gratings and 21 cells with contrast reversing gratings in 20 296 urethane-anaesthetised mice.

297

298 **Responses to drifting gratings**

299 Based on responses to drifting sinusoidal gratings, mouse V1 neurons 300 show the same separation into simple and complex cells as cats and primates 301 (Niell and Stryker, 2008). We classified cells as simple by the large modulation of 302 spiking rate $(F_1/F_0 > 1)$ to a drifting grating stimulus. The underlying membrane 303 potential of these neurons also exhibited large modulations when stimulated at 304 the preferred orientation, spatial frequency and temporal frequency (Fig. 3, 305 100% contrast). Membrane potential fluctuations were separated from spiking 306 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).

311 Previous work has demonstrated that the classification of simple cells 312 does not vary with contrast in the cat and primate (cat: Crowder et al., 2007; van 313 Kleef et al., 2010; monkey: Henry and Hawken, 2013; Cloherty and Ibbotson, 314 2015; Meffin et al., 2015). We first examined whether this is also true in mouse 315 V1 by measuring the changes in the modulation ratios for spiking rate (F_1/F_0) 316 and membrane potential (V_1/V_0) of individual simple cells with contrast (Fig. 317 4A). For simple cells, V_1/V_0 did vary with contrast but the F_1/F_0 was consistently 318 higher than unity, indicating that simple cell classification does not depend on 319 contrast (Fig. 4A). Across our sample population we found that the subthreshold 320 modulation ratio (V_1/V_0) of simple cells often increased with decreasing contrast 321 but this change was not statistically significant (n = 13, p > 0.05, one-sided t-test; 322 red symbols in Fig. 5A). This result is consistent with results from an earlier 323 study, in which simple cells in cat V1 showed increased V_0 and V_1 as contrasts 324 decreased (Carandini and Ferster, 1997). Despite those subthreshold changes, 325 however, the F_1/F_0 ratio was consistently above unity for simple cells at low and 326 high contrasts (Fig. 5B). Therefore, the simple cell population remains highly 327 phase sensitive at both the membrane potential and spiking output levels for all 328 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.,

332	2010; monkey: Henry and Hawken, 2013; Cloherty and Ibbotson, 2015; Meffin et
333	al., 2015). We found a range of contrast-dependent shifts in the modulation
334	ratios, which demonstrate that synaptic mechanisms are involved in this
335	process. For some complex cells modulations in response amplitude are clearly
336	evoked across all contrasts at the level of both the membrane potential and
337	spiking rate (Fig. 4B). Measures of the modulation ratios of the membrane
338	potentials systematically increase as contrast decreases. This shift is matched by
339	a commensurate increase in the F_1/F_0 ratios. For both spiking rate and
340	membrane potential responses, the mean responses (F $_0$ & V $_0$) dominate the
341	modulation amplitudes (F $_1$ & V $_1)$ at high contrasts (Fig. 4B). As the contrast
342	decreases, however, the differences between these two parameters declines and
343	results in increased modulation ratios. This trend is especially prominent in the
344	membrane potential responses in which V_1 remains largely unchanged compared
345	to V_0 . When considering the complex cell and the simple cell examples together,
346	it is noticeable that similar membrane potential characteristics in the two
347	example cells (Fig. 4A & B) are observed at higher contrasts (32% and 64%).
348	Both cells show substantial modulations of the fundamental frequencies of the
349	input (V ₁) that are well above the resting membrane potential, which result in
350	the V_0 component being larger than the V_1 component. However, the spiking
351	responses show different response characteristics in the two cells: cell A has a
352	larger F_1 component at high contrasts whereas cell B has a larger F_0 component
353	(Fig. 4A & 4B). As a result, cell A has an $F_1/F_0 > 1$ and is therefore classified as a
354	simple cell, whereas Cell B is classified as a complex cell because it has an F_1/F_0 <
355	1. These observations suggest that the dichotomy between simple and complex
356	cells based on spiking modulation ratios with high stimulus strengths does not

357 directly translate to corresponding distinctions in the membrane potential 358 responses. The differences in the F_1/F_0 ratios in the two cells are likely the result 359 of non-linear threshold transformations from the membrane potentials to the 360 spiking outputs (Priebe et al., 2004).

361 At the population level, changes in the F_1/F_0 (spiking rate) and V_1/V_0 362 (membrane potential) ratios of complex cells at high and low contrasts have 363 characteristics similar to the responses of the synaptic model. The scatter plots 364 of both F_1/F_0 and V_1/V_0 show significant increases at low contrast compared to 365 high contrast (Fig. 5). The distribution of V_1/V_0 ratios presented as a histogram 366 reveal a significant shift towards higher values at low contrasts compared to 367 high contrasts (n = 20, p = 0.008, one-sided t-test; blue symbols in Fig. 5A). The 368 population spiking responses also show significant increases in F₁/F₀ ratios at 369 low contrasts (n = 20, p = 0.02, one-sided t-test, Fig. 5B).

370

371 **Responses to contrast reversing gratings**

372 An alternative method to quantify the nonlinearities of cortical neurons is 373 to examine the modulated responses to contrast-reversing gratings (Hawken and 374 Parker, 1987). An ideal simple cell should modulate at the temporal frequency of 375 the contrast reversal (F_1) , and the timing of its response should depend on the 376 spatial phase of the grating (Fig. 6, left). An ideal complex cell should modulate 377 at twice the temporal frequency of the contrast reversal (F_2) , and the timing of its 378 response should not depend on the spatial phase of the grating (Fig. 6, right). 379 One can then distinguish simple and complex cells by considering the first and 380 second Fourier components in the complex plane (Fig. 6B). Simple cells should 381 have large F_1 components that lie along an axis in the complex plane. For the

382 example simple cell, that axis is along the abscissa. In contrast, the example 383 complex cell has small F_1 components, but a large F_2 component for which the 384 response does not change with the stimulus phase. To extract a metric that 385 describes the relative F₁ and F₂ modulations of the responses, we computed the 386 amplitude of the projection of the F_1 values onto their principle axis in the 387 complex plane, and compared that to the vector average F_2 value in the complex 388 plane. Doing so enforces the expectation that the timing of the F_2 component 389 should be invariant to spatial phase. The resulting contrast reversing modulation 390 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.

398 As with drifting gratings we found that reductions in contrast caused 399 systematic changes in membrane potential modulations that reflected a shift 400 toward more simple-like behaviour in complex cells. At high contrast these 401 complex cells are characterized by frequency doubled responses in both 402 membrane potential and spiking rate (Fig. 7A). When contrast was lowered, 403 however, the amplitude of the frequency doubled responses declined relative to 404 the modulation at the temporal frequency of the reversing grating. Note that not 405 only do modulations emerge at low contrasts, but the timing of the modulations 408 As shown in the membrane potential and spiking rate traces, the 409 projected F₂ and F₁ modulations vary across stimulus phase (Fig. 7B). To 410 quantify the changes outlined above for each cell, we estimated individual F_1 & 411 F₂ (spikes) and V₁ & V₂ (membrane potentials) values across all spatial phases 412 for each stimulus contrast tested. For F2 and V2, 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 in Fig. 6C and Fig. 7B). At high contrasts the amplitudes of the F_2 and V_2 416 417 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.

To quantify how contrast altered the behaviour of our complex cell population we estimated V_2/V_1 and F_2/F_1 modulation indices across our sample population (membrane potential: n = 21, spikes: n = 12; Fig. 8). We found that the membrane potential modulation ratio systematically declined with contrast, changing from a mean value of 0.83 to 0.57 (P = 0.016, one-sided t-test). There 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.

435 Discussion

436 Contrast-dependent phase sensitivity has been documented in a 437 subpopulation of neurons in the primary visual cortex of cat (Crowder et al., 438 2007; van Kleef et al., 2010; Hietanen et al., 2013) and primate (Henry and 439 Hawken, 2013; Cloherty and Ibbotson, 2015). The current study demonstrates 440 that contrast-dependent phase sensitivity is also present in the primary visual 441 cortex of mouse. Cortical visual processing in mice has been studied extensively 442 in the past decade (Niell and Stryker, 2008; Huberman and Niell, 2011; Tan et al., 443 2011). The abundant opportunities for genetic manipulation have made mouse 444 visual cortex a useful model in addition to carnivores and primates for studying 445 RF properties (e.g. Wang et al., 2006; Liu et al., 2009; Zariwala et al., 2011). All 446 previous literature showing contrast-dependent changes in response linearity in 447 cats and monkeys has been quantified using modulation ratios calculated from 448 extracellular responses to drifting sinusoidal gratings (Crowder et al., 2007; van 449 Kleef et al., 2010; Henry and Hawken, 2013; Hietanen et al., 2013; Cloherty and 450 Ibbotson, 2015) or contrast-reversing gratings (Meffin et al., 2015).

451 We used whole cell recordings to shed light on how complex cells emerge 452 in V1. A simple model to describe this process is that recurrent cortical 453 connectivity between neurons with distinct spatial selectivity generates the 454 spatial-invariant responses that characterize complex cells. Two models have 455 been proposed to describe this shift, one from Hubel and Wiesel in which simple 456 cells receive inputs from the dorsal lateral geniculate nucleus (dLGN) in the 457 thalamus and converge onto complex cells in one step to generate spatial 458 invariance (Hubel and Wiesel, 1962). Alternatively the generation of spatial 459 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).

We analysed the responses to drifting gratings and found that there is a shift in the input modulation ratio (V_1/V_0) with contrast consistent with a synaptic model (mean V_1/V_0 high contrast: 0.62, low contrast: 1.09). While the threshold nonlinearity may play a role in altering the phase sensitivity of neurons (Priebe et al., 2004), there is a clear synaptic component to the shift in phase sensitivity. Also, as expected, when stimulated with high-contrast reversing gratings these cells exhibited various degrees of frequency-doubled
responses (Meffin et al., 2015). However, at low contrasts, the same cells showed
more modulated, phase sensitive responses to drifting gratings and a tendency
to respond to selected spatial phases during stimulation with contrast-reversing
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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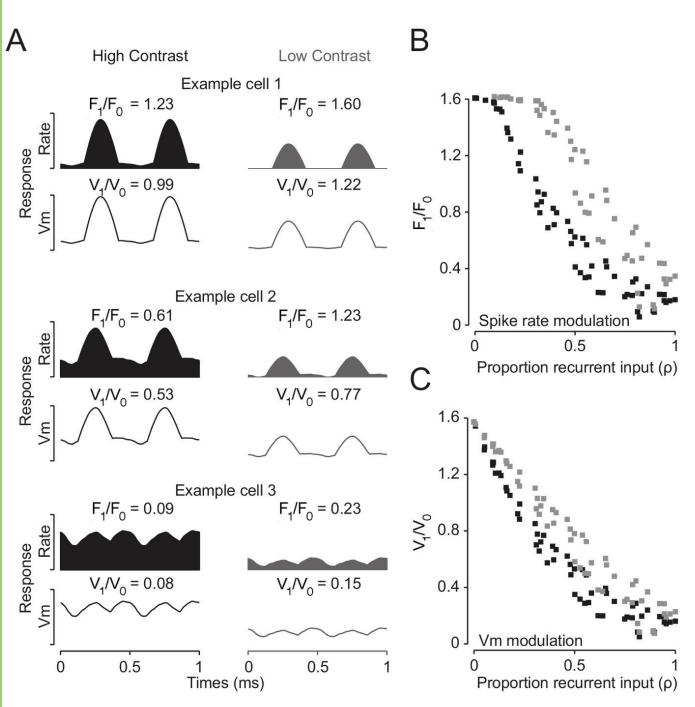
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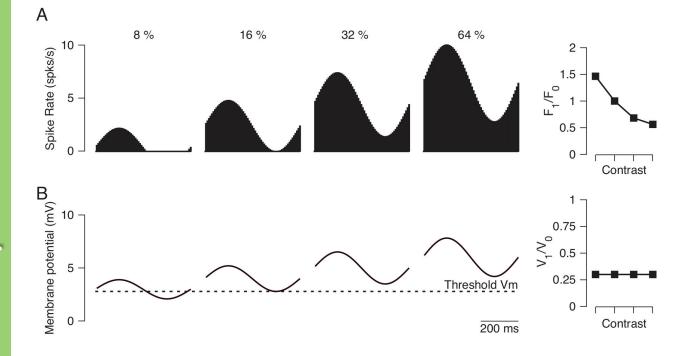
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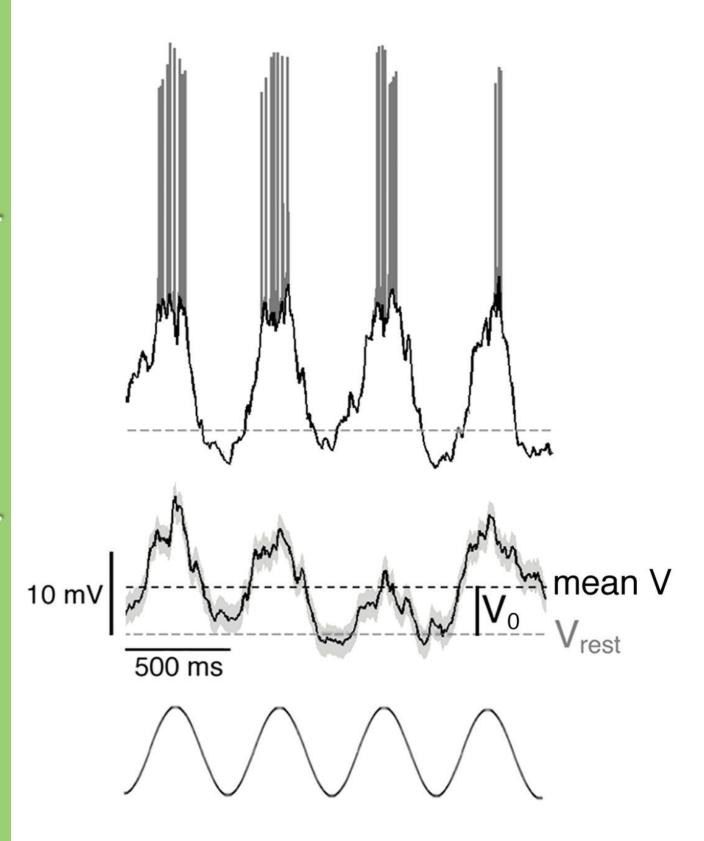
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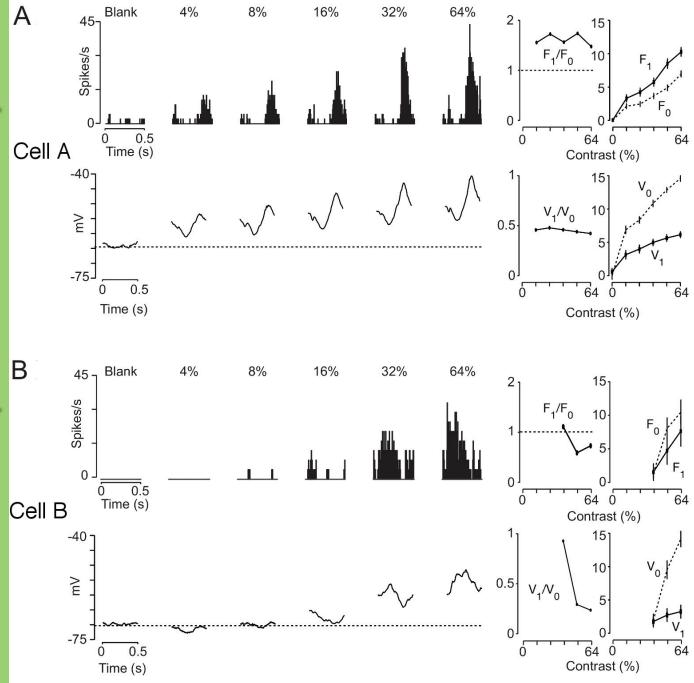




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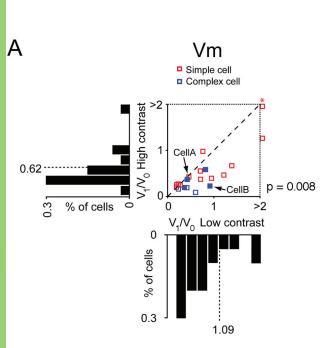


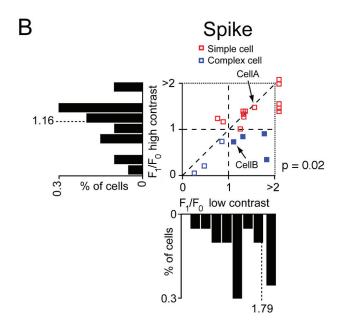


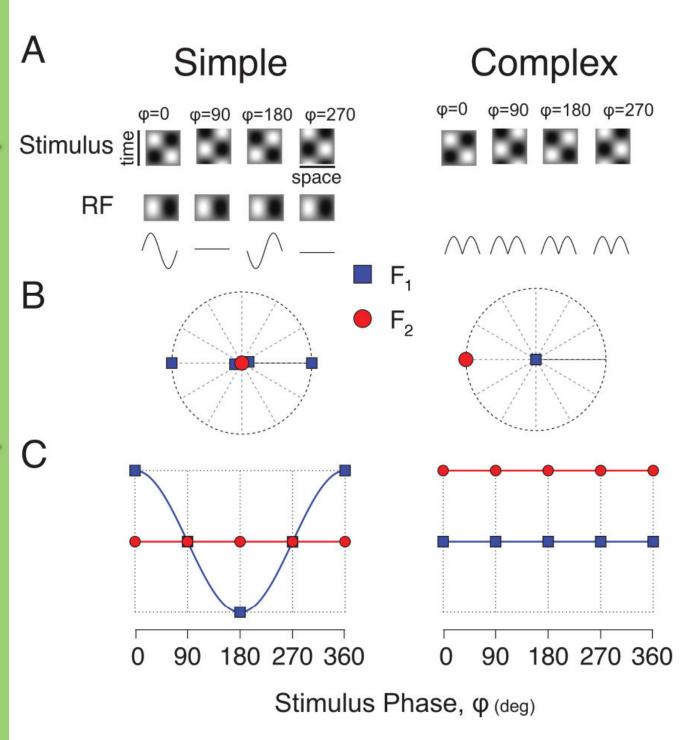


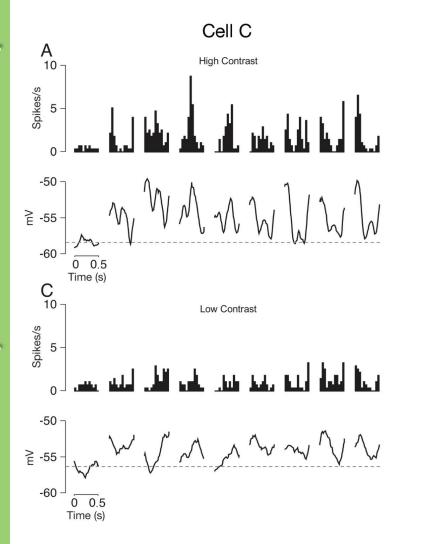
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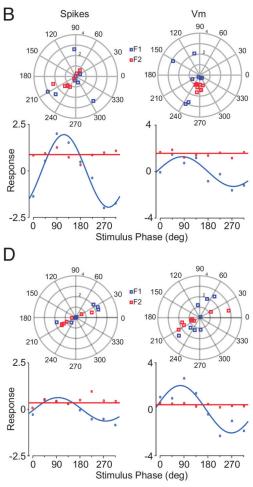












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