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PHASE SENSITIVITY OF COMPLEX CELLS IN PRIMARY VISUAL CORTEX

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Abstract—Neurons in the primary visual cortex are often classified as either *simple* or *complex* based on the linearity (or otherwise) of their response to spatial luminance contrast. In practice, classification is typically based on Fourier analysis of a cell's response to an optimal drifting sine-wave grating. Simple cells are generally considered to be linear and produce responses modulated at the fundamental frequency of the stimulus grating. In contrast, complex cells exhibit significant nonlinearities that reduce the response at the fundamental frequency. Cells can therefore be easily and objectively classified based on the relative modulation of their responses – the ratio of the phase-sensitive response at the fundamental frequency of the stimulus (F_1) to the phase-invariant sustained response (F_0). Cells are classified as *simple* if $F_1/F_0 > 1$ and *complex* if $F_1/F_0 < 1$. This classification is broadly consistent with criteria based on the spatial organisation of cells' receptive fields and is accordingly presumed to reflect disparate functional roles of simple and complex cells in coding visual information. However, Fourier analysis of spiking responses is sensitive to the number of spikes available – F_1/F_0 increases as the number of spikes is reduced, even for phase-invariant complex cells. Moreover, many complex cells encountered in the laboratory exhibit some phase sensitivity, evident as modulation of their responses at the fundamental frequency. There currently exists no objective quantitative means of assessing the significance or otherwise of these modulations. Here we derive a statistical basis for objectively assessing whether the modulation of neuronal responses is reliable, thereby adding a level of statistical certainty to measures of phase sensitivity. We apply our statistical analysis to neuronal responses to moving sine-wave gratings recorded from 367 cells in cat primary visual cortex. We find that approximately 60% of complex cells exhibit statistically significant ($\alpha < 0.01$) modulation of their responses to optimal moving gratings. These complex cells are phase sensitive and reliably encode spatial phase.

Key words: simple cells, response modulation, F_1/F_0, V1, vision.

INTRODUCTION

Two classes of cells, *simple* and *complex* (Hubel and Wiesel, 1962), have been identified in the primary visual cortices of all mammalian species studied to date (Skottun et al., 1991; Girman et al., 1999; Baker et al., 2001; Ibbotson et al., 2005; Niell and Stryker, 2008). This classification reflects the spatial arrangement of their receptive fields and the linearity of their response to spatial luminance contrast. Simple cells have spatially separate sub-regions within their receptive fields which evoke increased firing rates for light (ON) and dark (OFF) luminance contrast whereas complex cells have spatially overlapping light (ON) and dark (OFF) sub-regions. Simple cells combine responses to local stimuli presented within their receptive field approximately linearly while complex cells combine local responses in a highly nonlinear manner. By virtue of their receptive field organisation, simple cells preserve the sign of local stimulus contrast while complex cells do not (Movshon et al., 1978a; Skottun et al., 1991). Moreover, by virtue of their near linearity, the firing rate response of simple cells driven by moving sine-wave gratings are modulated in synchrony with the stimulus: their responses are phase sensitive (Movshon et al., 1978a,b,c; Skottun et al., 1991). In contrast, the canonical view holds that complex cells are phase invariant. When driven by optimal moving sine-wave gratings, complex cells' overlapping receptive field sub-regions and inherent nonlinearities reduce or even eliminate the response component at the fundamental stimulus frequency. This has lead to widespread use of Fourier analysis as a measure of relative modulation – the ratio of the amplitude of the phase-sensitive response at the fundamental frequency of the stimulus (F_1) to the amplitude of the phase-invariant sustained response (F_0) – as an objective means of classifying cortical neurons (Movshon et al., 1978a,b; Skottun et al., 1991). In striate cortices the discrepancy between classification based on relative modulation and that based on quantitative measures of receptive field organisation is estimated to be very small (Area 17/V1: Dean and Tolhurst, 1983; Skottun et al., 1991; Mata and Ringach, 2005; Bardy et al., 2006. Area

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Abbreviations: FFT, fast Fourier transform; LGN, lateral geniculate nucleus; SDF, spike density function.

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18/V2: Romo et al., 2011). Estimates of relative modulation therefore provide a viable means of inferring qualitative receptive field structure and quantifying phase sensitivity.

Here we demonstrate, using a stochastic model of an ideal phase-invariant complex cell, that estimating relative modulation (i.e., F_1/F_0) is sensitive to the number of spikes observed. Many investigators have noted that even when driven by optimal sine-wave gratings, some complex cells exhibit highly modulated responses together with a substantial sustained or mean response component (e.g., Mechler and Ringach, 2002). However, to date there exists no objective, quantitative method of assessing the reliability or otherwise of response modulations often observed in the laboratory. To this end we derive an analytic relationship for the dependence of F_1/F_0 on spike count and develop a sound statistical basis for assessing the reliability of estimates of F_1/F_0 in the laboratory. We then analyse spiking responses recorded under typical experimental conditions from 376 neurons in cat primary visual cortex (area 17 and 18). We find that when driven by optimal moving sine-wave gratings, a substantial proportion (~60%) of complex cells exhibit statistically reliable ($p < 0.01$) phase-sensitive response components modulated at the fundamental frequency of the stimulus. This has implications for models of visual processing in the primary visual cortex.

EXPERIMENTAL PROCEDURES

Modelling an ideal phase-invariant complex cell

Spiking responses of an ideal phase-invariant complex cell to an optimal moving sine-wave grating were modelled by assuming spike arrival times (t_i) to be independent identically distributed random variables uniformly distributed over the response interval. We defined the response interval to be one cycle of the stimulus grating such that $t_i \in [-\pi, \pi)$, $i = 1 \ldots n$, where n is the number of spikes (proportional to the response spike rate). This model makes no attempt to model the spatio-temporal tuning of the cell, stimuli are assumed to be optimal moving sine-wave gratings. Similarly, no attempt is made to model the biophysics of spike generation (refractory period etc.).

Simulated spiking responses ($r(t)$) were expressed as a sum of delta functions,

$$r(t) = \sum_{i=1}^{n} \delta(t - t_i) \quad (1)$$

We then calculated the relative modulation (F_1/F_0) of the response by taking the ratio of the amplitude of the response component modulated at the fundamental frequency of the stimulus (F_1) and the amplitude of the sustained response (F_0). The amplitudes of the sustained and modulated components of the response (F_0 and F_1) were given by the first two terms of the Fourier series expansion of $r(t)$ (for details, see the Appendix). For each simulated spike count (n) this process was repeated 100,000 times to generate an empirical distribution of F_1/F_0. From this distribution we calculate $\langle F_1/F_0 \rangle$, the expected value of F_1/F_0 for the given spike count (n). This simple model reveals the dependence of $\langle F_1/F_0 \rangle$ upon the number of spikes (n) available. We then compare F_1/F_0 values from experimentally observed responses to the corresponding probability distribution based on the number of spikes collected. Cells whose measured F_1/F_0 values lay outside the bottom 99% of their associated probability distribution were considered to have a statistically significant F_1 component.

Anaesthesia and surgical procedures

Extracellular recordings of spiking responses were made from single units in the primary visual cortex (area 17 and 18) in anaesthetized cats ($n = 19$; 2–5 kg), as described previously [3, 7, 21, 46]. All experiments were performed in accordance with the National Health and Medical Research Council's Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved either by the Animal Experimentation Ethics Committee of the Australian National University (R.VS.20.05) or the Animal Care Ethics Committee of the University of Sydney (KO3/3-2008/1/4673 & KO3/3-2008/3/4673).

In Sydney, animals were initially anaesthetised by inhalation of 2–4% isoflurane (Abbott Australasia Pty Ltd, Kurnell, NSW, Australia) in a 2:1 mixture of N_2O and O_2. In Canberra, animals were initially anaesthetised by intramuscular injection of ketamine hydrochloride (20 mg/kg; i.m.) and xylazine (1 mg/kg). A suitable depth of anaesthesia was determined by absence of the toe pinch reflex. Animals were then intubated to ensure adequate respiration and the right cephalic vein was cannulated. Animals were then placed in a stereotaxic frame and anaesthesia was maintained for the duration of the experiment by inhalation of gaseous isoflurane (0.75–1.5% during surgery, 0.5% during unit recordings; Sydney) or halothane (1–2% during surgery, 0.5% during unit recordings; Canberra) in a 2:1 mixture of N_2O and O_2. Animals were instrumented to facilitate continuous monitoring of the electrocardiogram (ECG), the electroencephalogram (EEG) and end-tidal CO_2 concentration to ensure an adequate level of anaesthesia was maintained at all times. Changes in the physiological indicators (ECG, EEG or expired CO_2) that may have suggested the level of anaesthesia was not sufficient were mitigated by immediately increasing the concentration of inhaled isoflurane or halothane. For fluid replacement, animals received a continuous intravenous infusion (2.5 ml/kg/h) 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 37.7 °C by way of an electric heating blanket under feedback control.

To allow access to the primary visual cortex (area 17 and 18), the scalp was reflected and a craniotomy was performed 0–8 mm posterior to interaural zero and 2–8 mm lateral to the midline. To minimise eye movements during single unit recordings animals were subject to neuromuscular blockade by intravenous injection of 50 mg of gallamine triethiodide (Flaxedil; Sigma, St. Louis, MO) in 2 ml of Hartmann's solution. Blockade was then maintained by continuous intravenous infusion of Flaxedil at a rate of 10 mg/kg/h. Animals were mechanically ventilated to maintain end-tidal CO_2 between 3.5% and 4%.

The pupils were dilated and the nictitating membranes retracted by topical application of ophthalmic atropine sulphate (1%) and phenylephrine hydrochloride (2.5%). 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. Spherical and chromatic aberrations were minimised by interposing 3-mm diameter artificial pupils between the eyes and the corrective lenses. Animals received daily injections to reduce salivation (atropine, 0.2 mg/kg; s.c.), cerebral oedema (dexamethasone phosphate, 1.5 mg/kg; i.m.) and the risk of infection (Clavulox, a broad spectrum antibiotic, 0.5 ml/kg; i.m.).

At the conclusion of the experiment animals were killed by intravenous injection of an overdose of barbiturate (sodium pentobarbitone, 150 mg/kg) and immediately transcardially perfused with 0.9% saline followed by 10% formol saline. The brain was then extracted for histological reconstruction of recording track locations (for details see Crowder et al., 2006).