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Spatiotemporal tuning in mouse primary visual cortex

E.E. LeDue, M.Y. Zou, N.A. Crowder*

Department of Psychology and Neuroscience, Dalhousie University, Halifax, Nova Scotia B3H 4R2, Canada

HIGHLIGHTS

- We investigated spatiotemporal tuning in mouse primary visual cortex.
- We quantified speed tuning of individual V1 neurons using established metrics.
- Mouse V1 neurons as a population are not speed tuned.

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ABSTRACT

The neural correlates of visual motion perception have historically been studied in non-human primates. However, the mouse has recently gained popularity as a model for studying vision primarily driven by the hope that the genetic tools available in this species may contribute to our understanding of visual processing in the cortex. A recent calcium-imaging study on the spatiotemporal tuning of mouse striate and extrastriate cortex revealed that neurons in the primary visual cortex (V1) were almost never speed tuned, whereas previous electrophysiological studies in macaques noted around one quarter of V1 neurons appeared to be selective for a particular stimulus speed. We were interested in whether this discrepancy was due to methodological or species differences, so we measured the spatiotemporal tuning of mouse V1 neurons using standard electrophysiological techniques. Using comparable analyses to previous studies of speed tuning, our data showed that speed tuning is rare in mouse V1, which corroborates earlier studies in mouse and points to a species difference in motion processing in early cortex between macaques and other mammals.

1. Introduction

Visual motion appears to be a salient and biologically relevant visual stimulus for many animals [1,11]. In behavioral tasks, human and non-human primates are capable of extracting the speed of a moving stimulus from the visual array [3,4,14,29], and humans are capable of discerning variations in stimulus speed of <5% [17]. Any moving visual stimulus can be decomposed into sine wave gratings of various spatial frequencies (SF) and temporal frequencies (TF), and the neural processing of stimulus speed can be tested in the laboratory using drifting sinusoidal gratings as simplified stimuli because a grating's speed is calculated as the ratio of its TF and SF. In macaques, studies in visual cortex and specialized motion processing areas like the middle temporal area (MT) have revealed that neuronal responses to these sine wave stimuli can be classified as either inseparable or separable: inseparable neurons have a constant preferred speed over many SFs, which usually manifests as an elongated peak in the spatiotemporal domain that is oriented diagonally along an iso-speed line (hence tuning for SF and TF cannot be separated); separable neurons have a preferred SF and their preferred speed varies with TF [24–26]. Around 25–60% of MT neurons, and 25% of V1 complex neurons have been found to be speed selective in macaques [24–26].

Behavioral studies have shown that mice, like primates, are sensitive to stimulus speed [33]. Recently, mice have become a popular model of cortical visual processing because of the availability of genetic tools that allow for manipulation of different cell types or circuits that are important for vision. Despite the poor visual acuity of the mouse, their V1 shares many similarities with primates, such as SF tuning, orientation tuning, and the presence of both simple and complex cells [10,21]. The small size and lissencephalic structure of the mouse cortex could also be viewed as advantageous, as it allows easy simultaneous study of striate and extrastriate visual areas using electrophysiology or imaging techniques. It is possible that mouse models could provide us with insight into the neural processing of motion coding and speed tuning, but comparisons to existing research in other species must first be established.

* Corresponding author at: Department of Psychology and Neuroscience, Dalhousie University, 1355 Oxford Street, PO Box 15000, Halifax, Nova Scotia, Canada B3H 4R2. Tel.: +1 902 494 6025; fax: +1 902 494 6585.
E-mail address: nathan.crowder@dal.ca (N.A. Crowder).

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Andermann et al. [2] were the first to investigate speed tuning in mouse striate and extrastriate areas using *in vivo* two-photon calcium imaging in awake animals and found that unlike macaque V1 very few neurons in mouse V1 were speed selective, and that other extrastriate areas were responsible for processing of stimulus speed. Although calcium imaging allows data collection from huge populations of individual neurons simultaneously, it is an indirect measure of spiking rate, so there are several caveats in interpreting this data. First, the fluorescence of both synthetic and genetically coded calcium indicators is non-linear, and saturation creates difficulty in interpreting very high or low spike rates [32], although the GCAMP3 indicator used by Andermann et al. [2] suffers less from these problems. Second, calcium imaging is currently limited to the superficial layers of the cortex. We were interested in whether the difference between macaque V1 and mouse V1 neurons might be due to methodological differences, as speed tuning in macaque V1 was measured using electrophysiology in anesthetized animals.

In this study, we recorded single-unit responses in anesthetized mice to drifting sine wave gratings for 36 combinations of SFs and TFs to obtain spatiotemporal response profiles. To evaluate the magnitude of speed tuning for each unit we fit our data with a two-dimensional Gaussian following the methods used in macaque studies [25], and calcium imaging studies in mouse [2]. Our results were also re-analyzed with a partial correlation analysis following Priebe et al. [25]. We found that inseparable tuning was rare in mouse V1, which agrees with earlier data obtained with two-photon calcium imaging [2].

2. Materials and methods

2.1. Animals and surgical preparation

Ethics conformed to Canadian Council on Animal Care guidelines and were approved by the University Committee for Laboratory Animals at Dalhousie University. Recordings were made from 17 adult male C57BL/6J mice ranging from 20 to 30 g in weight (purchased from the Jackson Laboratory). Mice were anesthetized with urethane (0.5 g/kg ip, Sigma Aldrich, St. Louis, MO), and body temperature was maintained at 37.5 °C with a heating pad. If needed, a small dose of ketamine (20 mg/kg ip) was given to accelerate descent to the surgical plane of anesthesia [18]. A tracheotomy was performed prior to securing mice in a stereotax [18]. Mice were left free-breathing throughout the experiment and a tube located in front of the mouse delivered oxygen (0.1 L/min) to supplement room air. Additional urethane doses were given as required, and chlorprothixene (5 mg/kg ip, Sigma Aldrich) was also given with the first urethane top up. A craniotomy (~1 mm^2) was made 0.8 mm anterior and 2.3 mm lateral to lambda [22], which corresponds to the primary visual cortex in mice. Recordings were made using either glass (2–5 μm tip diameter, filled with 2 M NaCl) or carbon fiber in glass microelectrodes (0.6–1.5 MΩ impedance). Electrode depth was controlled using a micromanipulator (FHC, Bowdoin, ME). Extracellular signals from individual units were amplified (Xcell 3+, FHC) and filtered (bandpass: 50–2000 Hz) before being digitized (Cambridge Electronic Design Power1401 with Spike2, Cambridge, England). Acquired signals were sampled at 40 kHz, and online analysis was performed on triggered TTL pulses with Spike2, but subsequent analysis was done offline.

2.2. Visual stimuli

Receptive field locations for visually responsive units were initially mapped out by hand using a light bar, then quantitatively characterized online for orientation selectivity and surround suppression using drifting square wave gratings. Spatiotemporal tuning was assessed with drifting sine wave gratings with 36 combinations of SFs (0.01, 0.02, 0.04, 0.08, 0.16 and 0.32 cpd) and TFs (0.25, 0.5, 1, 2, 4, 8 Hz). All spatiotemporal stimuli were presented at the optimal orientation and size for each unit. Presentations of different spatiotemporal combinations were randomized with 8–10 repeats for each combination. The presentation time of the stimulus was 1.5 s, and a gray of mean luminance was shown between stimuli for 0.5 s. Grating start-phase was staggered on each repetition to average out periodic firing of phase-sensitive neurons. Viewing distance depended on the location of the receptive field and was adjusted on a unit-to-unit basis, but ranged from a distance of 15–35 cm. All units were from the monocular representation of V1 (~30°–100° lateral to the vertical meridian) [22,30]. All stimuli were custom made using the Psychophysics toolbox extension for Matlab (Mathworks, Natick MA) [5,23], and presented on a calibrated CRT monitor (LG Flatron 915FT plus 19", 100 Hz refresh rate, 1024 × 768 pixels, mean luminance = 30 cd/m^2).

2.3. Data analysis

Spike sorting was performed with Spike2, and a principle components analysis was used to isolate single units. For each unit, we calculated the magnitude of orientation and size tuning using a discrimination index (DI) [6]:

$$\text{Discrimination index} = \frac{(Resp_{Max} - Resp_{Min})}{((Resp_{Max} + Resp_{Min}) + 2\sqrt{SSE}/(N - M))} \quad (1)$$

$Resp_{Max}$ is the neuron's max response, while $Resp_{Min}$ is the neuron's minimum response. *SSE* is the sum of squared error of the mean, *N* is the total number of presentations of the stimuli, and *M* is the number of different stimuli presented.

In order to classify cells as simple or complex, we divided the first Fourier coefficient of a neuron's response to a grating at the spatiotemporal peak (F_1) by the mean time-averaged response to this grating (F_0). The F_1/F_0 ratio has been used to quantitatively classify simple and complex cells [19,20,28,31], and an F_1/F_0 ratio less than 1 indicates a cell is complex.

In order to quantify speed tuning in individual neurons we fit spatiotemporal responses to a two-dimensional Gaussian using the least squares method (following the fitting methods used by Priebe et al. [25], and also by Andermann et al. [2]):

$$R(sf, tf) = A * \exp \frac{-(\log_2(sf) - \log_2(sf_0))^2}{\sigma_{sf^2}} * \exp \frac{-(\log_2(tf) - \log_2(tf_p(sf)))^2}{\sigma_{tf^2}} \quad (2)$$

where *A* is the peak response, sf_0 is the neuron's preferred spatial frequency and tf_p is dependent on the preferred spatial frequency, as defined by:

$$tf_p(sf) = 2^{(\xi)*(\log_2(sf) - \log_2(sf_0)) + \log_2(tf_0)} \quad (3)$$

The parameter ξ defines the slope of the relationship between the preferred TF and SF. If a neuron is inseparable, its preferred speed will remain constant as the SF of the stimulus is altered, and ξ will be around 1. In contrast, if a neuron is separable, its preferred speed changes as SF changes, and ξ will be approximately 0. A secondary analysis that also addressed speed tuning used partial correlations to test whether a neuron's tuning was more highly correlated with a model in which ξ was constrained to either 0 or 1, which represented complete separability or perfect speed tuning,