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Effects of stimulus spatial frequency, size, and luminance contrast on orientation tuning of neurons in the dorsal lateral geniculate nucleus of cat☆

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ABSTRACT

It is generally thought that orientation selectivity first appears in the primary visual cortex (V1), whereas neurons in the lateral geniculate nucleus (LGN), an input source for V1, are thought to be insensitive to stimulus orientation. Here we show that increasing both the spatial frequency and size of the grating stimuli beyond their respective optimal values strongly enhance the orientation tuning of LGN neurons. The resulting orientation tuning was clearly contrast-invariant. Furthermore, blocking intrathalamic inhibition by iontophoretically administering γ-aminobutyric acid $(GABA)_A$ receptor antagonists, such as bicuculline and GABAzine, slightly but significantly weakened the contrast invariance. Our results suggest that orientation tuning in the LGN is caused by an elliptical classical receptive field and orientation-tuned surround suppression, and that its contrast invariance is ensured by local $GABA_A$ inhibition. This contrast-invariant orientation tuning in LGN neurons may contribute to the contrast-invariant orientation tuning seen in V1 neurons.

1. Introduction

For more than half a century, it has been generally thought that orientation selectivity first emerges in the primary visual cortex (V1), where it offers important insight on the thalamocortical transformation of sensory inputs (Hubel and Wiesel, 1959, 1962). However, several studies have reported that a certain population of neurons in the lateral geniculate nucleus (LGN) exhibits moderate orientation-biased responses to stimulation with high spatial-frequency gratings (Ahmed and Hammond, 1991; Shou et al., 1995; Shou and Leventhal, 1989; Soodak et al., 1987; Suematsu et al., 2012; Thompson et al., 1994; Vidyasagar, 1984; Vidyasagar and Urbas, 1982; Xu et al., 2002; Zhou et al., 1995). In those studies, the orientation-biased response of the LGN neurons was explained by an anisotropy that describes an elliptical classical receptive field (CRF) center (Ahmed and Hammond, 1991; Passaglia et al., 2002; Soodak et al., 1987) that is thought to originate from retinal ganglion cells. This is because of evidence for retinal ganglion cells also having an elliptical CRF center (Hammond, 1974; Suematsu et al., 2012) based on their asymmetrical dendritic morphology (Shou et al., 1995) and reports showing they exhibit orientation-biased responses when tested with high spatial-frequency gratings (Levick and Thibos, 1980; Soodak et al., 1987; Suematsu et al., 2012).

Additionally, LGN neurons receive non-linear response modulation from the CRF surround (Bonin et al., 2005; Ishikawa et al., 2010; Naito et al., 2007; Ozeki et al., 2004; Sadakane et al., 2006; Solomon et al., 2002; Webb et al., 2002; Webb et al., 2005), an area often referred to as the extraclassical receptive field (ECRF). Responses to CRF stimulation are usually suppressed by concurrent activation of the ECRF, the modulation of which is called surround suppression. Several studies have reported that such modulation effects on LGN neurons are significantly orientation-tuned (Jones and Sillito, 1994; Naito et al., 2007; Suematsu et al., 2012; Sun et al., 2004; Vidyasagar and Urbas, 1982). Furthermore, the degree of surround

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suppression in LGN neurons significantly weakens with decreasing stimulus luminance contrast (Bonin et al., 2005; Ozeki et al., 2004; Sadakane et al., 2006; Solomon et al., 2002). These results suggest that not only the stimulus spatial frequency, but also the stimulus size and luminance contrast may have significant effects on the orientation tuning of LGN neurons.

In this study, to investigate the magnitude of orientation selectivity in LGN neurons and the mechanisms involved, we examined the effects of spatial frequency, stimulus size, and the luminance contrast of grating stimuli on the orientation tuning of LGN neurons. We found that increasing both the spatial frequency and size of the gratings significantly enhances the orientation selectivity, while changes in the luminance contrast exhibited little or no effect on the degree of orientation tuning. Furthermore, blocking intrathalamic inhibition by iontophoretic administration of bicuculline or GABAzine, two $GABA_A$ receptor antagonists, substantially weakens the orientation tuning in a luminance contrast-dependent manner and diminishes the contrast invariance, particularly at high contrast.

2. Materials and methods

All procedures were performed in accordance with the National Institute of Health guidelines for the care of experimental animals and approved by the Animal Care Committee of the Medical School of Osaka University (Permit number: 20-149-0). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.1. Animal preparation

Details of the experimental preparation are described elsewhere (Akasaki et al., 2002; Naito et al., 2007). Eighteen adult cats weighing 2.5–4.0 kg were anesthetized with ketamine (10 mg/kg, i.m.) followed by a mixture of isoflurane (2–3%) and N_2O:O_2 (2:1). The animals were continuously paralyzed with pancuronium bromide (0.1 mg/kg/h, i.v.) and maintained under artificial ventilation. During the recording of neuronal activity, isoflurane was decreased to 0.3–1.0% in N_2O:O_2 (2:1) and fentanyl citrate (Fentanest; Sankyo, Tokyo, Japan; 10 μg/kg/h, i.v.) was continuously infused. The rectal temperature and end-tidal CO_2 concentration were adjusted to 37–38 °C and 3.5–4%, respectively. The electrocardiogram, electroencephalogram and heart rate were continuously monitored throughout the experiment. The nictitating membrane was retracted and the pupil was dilated by topical administration of a mixture of tropicamide (0.5%) and phenylephrine hydrochloride (0.5%) (Mydrin-P, Santen, Osaka). Each cornea was protected with an O_2 permeable contact lens with an artificial pupil (4 mm in diameter).

2.2. Visual stimulus

Drifting sinusoidal grating was monocularly presented on a monitor (CPD-G500J, SONY; mean luminance, 40 cd/m^2; screen size, 40 cm × 30 cm; resolution, 1024 × 768 pixels; and refresh rate, 100 Hz), generated by VSG 2/3 (Cambridge Research System, UK), and controlled by an IBM-PC/AT-compatible computer. The monitor was placed 57 cm from the cat's eyes and focused on the retina.

2.3. Extracellular recording

Tungsten-in-glass microelectrodes or multibarrel glass microelectrodes were used for extracellular single unit recordings and neuropharmacology (multi-barrel glass microelectrodes only) in the LGN. Electrophysiological signals were amplified and filtered (low and high cutoff frequencies were 300 and 5000 Hz, respectively) using an AC amplifier (Model 1800; AM Systems, USA) and sent to a spike sorter (Multi-Spike Detector; Alpha Omega Engineering, Nazareth, Israel), which performed on-line template matching of the action potentials. Digital pulses obtained by the template matching were acquired using a time-stamping board (Lisberger Tech., San Francisco, USA) at a sampling rate of 10 kHz. Single-unit action potentials were identified by the spike waveform with template matching. To eliminate the possibility of multi-unit activities, we conducted the following. First, the presence of a refractory period was confirmed in the auto-correlogram of each neuron (Okamoto et al., 2009). We also counted the number of spikes with interspike intervals less than 1 ms. If these exceeded 1% of the total spikes for a given cell during the entire recoding period, data for the cell were discarded (Okamoto et al., 2009). Finally, we used only the neurons whose waveforms were stable throughout the entire recording period.

Cells were recorded from the area-centralis or near area-centralis (within ∼15°) of the LGN and classified as X or Y type by employing commonly used criteria (Cleland et al., 1971; Enroth-Cugell and Robson, 1966; Hochstein and Shapley, 1976). The F1 component of the response to a drifting grating (2 s) was used as the response magnitude. Each neuron's receptive field was initially characterized by its tuning for location, diameter, orientation, spatial frequency, temporal frequency, and luminance contrast (Michelson contrast) of the drifting gratings. We measured orientation-tuning curves (0–337.5°; 22.5° steps) with a drifting circular grating patch (21.3° in diameter) at several spatial frequencies. Once an orientation-biased response was observed, the preferred orientation and orthogonal orientation was determined by the tuning. The spatial frequency that elicited maximum response at optimal orientation was adopted as the optimal spatial frequency. The highest spatial frequency that elicited at least 50% of the optimal spatial frequency response was chosen as the high spatial frequency. The optimal stimulus size was determined by spatial-summation tuning (0.1–21.3° in diameter), which elicited the maximum response at the preferred orientation at optimal spatial frequency. Finally, orientation tunings were measured again at each spatial frequency and size. For a subpopulation of neurons, we measured spatial-summation tuning at optimal and high spatial frequencies in the preferred orientation and that orthogonal to the preferred (orthogonal orientation).

2.4. Procedure for pharmacological experiments

Multi-barrel glass micropipettes were used for extracellular single-neuron recordings and for the iontophoretic administration of bicuculline methiodide (BMI; Sigma, St. Louis, MO; 5 mM, pH 4.0) (Ozeki et al., 2004) and SR-95531 (GABAzine; Tocris Bioscience, UK, MO; 10 or 20 mM, pH 4.4) (Katzner et al., 2011). The tip of the recording pipette protruded 20–30 μm from that of the drug pipette. The ejecting and retaining BMI currents were between +5 and +60 nA and between −10 and −20 nA, respectively. The ejecting and retaining GABAzine currents were between +20 and +100 nA and between −10 and −20 nA, respectively. During BMI or GABAzine administration, all neurons exhibited significant increases in mean response amplitude at the highest contrast condition used by a factor of 2.72 ± 0.88 (1.10–4.08; N = 20) and 1.98 ± 0.60 (1.15–3.41; N = 12) relative to the control response, respectively. All errors in the manuscript represent standard deviations (SD).

2.5. Laminar analysis

At the end of each penetration, three to four electrolytic lesions (DC currents; 3–4 μA for 5–10 s; tip negative) were made for the