Summary of doctoral thesis

State-Dependent Changes in Spatial Frequency Tuning in the Primary Visual Cortex

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Introduction

Brain states dramatically influence the processing of sensory information. At high arousal levels, such as during attention, the acuity to perceive visual stimuli is improved. To understand the neuronal mechanisms of state-dependent perceptual modulation, it is crucial to elucidate the state-dependent changes in visual responses to incoming stimuli. Several studies have compared the visual response properties in the primary visual cortex (V1) between the awake and anesthetized states. The gain of visual responses, rather than visual response selectivity, is modulated by the brain states in the mouse V1. Orientation preference and the tuning of sharpness in V1 neurons are not altered based on the state. Although spatial frequency (SF) is one of the fundamental visual characteristics, the state-dependent modulation of SF tuning properties has not been comprehensively explored. Moreover, compared with excitatory neurons, the state-dependent changes in the activity of inhibitory neurons have been less reported.

In this study, I investigated the differences in SF tuning properties between anesthetized and awake states in mouse V1. Using *in vivo* two-photon Ca^{2+} imaging, individually recorded neurons in the presumed layer 2/3 were matched across

imaging sessions under awake and anesthetized conditions while identifying the neuron types. This enabled me to directly compare the visual responses recorded from the same neurons between the two states.

Materials and methods

All experimental procedures were approved by the Experimental Animal Committee of the National Institute for Physiological Sciences. Mice of either sex at the age of 2–5 months (C57BL/6 background) were used for two-photon Ca^{2+} imaging. Vgat-IRES-Cre mice (B6J.129S6(FVB)-Slc32a1$^{tm2(cre)Lowl}$/MwarJ, The Jackson Laboratory, stock #16962) were crossed with Ai14 tdTomato reporter mice (B6;129S6-Gt(ROSA)26Sor$^{tm14(CAG-tdTomato)Hze}$/J, The Jackson Laboratory, stock #007908) to label inhibitory neurons. To express Ca^{2+} indicator in excitatory and inhibitory neurons, AAV1-Syn-jGCaMP7b-WPRE (Addgene) was injected into the primary visual cortex of the mice. For the injection of the AAV vector, mice were anesthetized with medetomidine (0.75 mg/kg; Domitor, Zenoaq), midazolam (4.0 mg/kg; Midazolam sand, Sandoz), and butorphanol (5.0 mg/kg; Vetorphale, Meiji Seika) by intraperitoneal injection.

Calcium imaging was performed using a two-photon microscope (Nikon A1MP, Tokyo, Japan). Images were acquired using a 25x water-immersion objective (MRD77220, 1.10 NA, Nikon). GCaMP7b and tdTomato were excited at 950 nm using 500/50 nm and 663/75 nm emission filters, respectively. The imaging depth was set at 180–300 μm below the pia (presumed layer 2/3). Mice were first imaged in the awake condition and then imaged under light anesthesia using isoflurane

(0.6–0.8%) in air. Each imaging session lasted approximately 50 min. In some experiments, an additional awake session was conducted after recovery from anesthesia in the home cage for at least 4 h.

To evoke visual responses, full-screen sine-wave drifting gratings (100% contrast, 100 cd/m^2 mean luminance) using combinations of six SFs (0.02–0.64 c/d, in one-octave steps) in eight directions (0–315°, in 45° steps) at a fixed temporal frequency (2 Hz) were presented to the mouse. To estimate the SF tuning property, the average stimulus-evoked responses were fitted to a two-dimensional Gaussian model. The tuning parameters were determined based on the fitted curves in the anesthetized and awake conditions. The sharpness of the SF tuning was assessed by the full width at half maximum of the SF tuning curve in the optimal direction.

Results

I studied the effects of brain state on SF tuning in excitatory and inhibitory neurons residing in the presumed layer 2/3 of mouse V1. Using two-photon Ca^{2+} imaging, I compared SF tuning properties in the same neurons recorded under light isoflurane anesthesia and awake conditions. To analyze excitatory and inhibitory neurons separately, I labeled inhibitory neurons with tdTomato using Vgat-Cre×Ai14 mice. Both excitatory and inhibitory neurons expressed GCaMP7b, which was used to measure Ca^{2+} activity. In all nine mice, visual responses were first recorded in the awake conditions and then recorded in anesthetized conditions. Furthermore, in five of the nine mice, the responses were also recorded in the recovery condition in which mice were fully recovered from anesthesia.

I first analyzed visual responses from excitatory neurons. I observed that in a subset of excitatory neurons, the preferred SFs were significantly shifted to higher frequency values during the awake state. Linear mixed-effect models were used to assess the relationship of preferred SFs between awake and anesthetized conditions. Anesthesia reduced the preferred SFs in excitatory neurons. The sharpness of the SF selectivity was assessed by the full width at half maximum of the SF tuning curve in the optimal direction. The width was significantly narrower in the awake state than in the anesthetized state, demonstrating that SF selectivity became sharper in the awake state. Orientation and direction selectivity were assessed for the preferred SFs. There were no significant state-dependent differences in orientation selectivity. In addition, direction selectivity was not significantly different between awake and anesthesia conditions. These results about the state dependence on orientation and direction selectivity are consistent with the findings of previous studies.

To characterize other visual response properties in SF-shifted neurons, state-dependent changes in tuning sharpness were examined in SF-shifted and unshifted neurons separately. In both SF-shifted and unshifted neuron groups, the SF selectivity was higher in the awake state than in the anesthetized state. In SF-unshifted neurons, the strength of maximal visual responses significantly decreased in the awake state. However, the strength did not decrease significantly based on the brain state in the shifted neurons. Therefore, SF-shifted neurons can exhibit relatively strong responses to higher SF stimuli in the awake state.

To verify if the observed changes in SF tuning resulted from a recording-sequence effect, SF tuning properties were compared between the first awake session and the recovered condition from anesthesia (the second awake session) in a subset

of animals (five mice). The preferred SFs of individual neurons did not significantly change between the first and second awake sessions. Therefore, it is unlikely that the difference in SF tuning properties between the awake and anesthetized states was due to a recording sequence effect.

Subsequently, I analyzed the state-dependent changes in SF tuning in inhibitory neurons. I identified inhibitory neurons according to tdTomato expression. Most inhibitory neurons were tuned to higher SFs in the awake condition than under anesthesia, similar to excitatory neurons. These data demonstrated that both excitatory and inhibitory neurons preferred higher SF stimuli in the awake state.

Discussion

The state-dependent changes in visual response selectivity were investigated in the presumed layer 2/3 of mouse V1. Using two-photon Ca^{2+} imaging, I matched individual neurons across sessions under awake and anesthesia conditions, enabling direct comparison of visual responses recorded from the same neurons between these two states. I observed that in a subset of excitatory neurons, the preferred SFs were shifted to higher frequency values during the awake state. My results support the notion that visual response properties of awake mice differ from those of anesthetized mice. Notably, inhibitory neurons also exhibited a state-dependent shift in preferred SFs. These results suggest that in neurons within layer 2/3, the preferred SFs became higher in the awake state irrespective of neuron type.

I also found that SF selectivity was sharpened in excitatory neurons in the awake state. However, the orientation and direction selectivity remained unchanged.

Previous studies demonstrated that orientation and direction selectivity in layer 2/3 of mouse V1 does not change depending on the brain state, although a previous study reported direction selectivity changes. In addition, it has been reported that the preferred orientation remains unchanged between awake and anesthetized states or between resting and locomotion states in mouse V1. Therefore, SF preference and tuning in layer 2/3 excitatory neurons of V1 appear to be highly modifiable by the brain state, compared to other visual parameters.

Psychological studies have reported that behavioral states strongly modulate the spatial resolution of sensory perception. Attention improves the spatial acuity of vision. There is evidence for state-dependent modulation of visual response properties related to spatial patterns in mouse V1. Neurons tuned to high SFs exhibit a larger relative gain compared with those tuned to lower spatial frequencies during locomotion than during rest. Anesthesia strongly suppresses the tuning of stimulus size. My findings demonstrate that wakefulness induces a shift of preferred SFs to higher frequency values. These changes in spatial information representation may contribute to state-dependent changes in the acuity of visual perception.