MODULATION OF THE AMPLITUDE OF GAMMA-BAND OSCILLATIONS BY STIMULUS PHASE IN MOUSE VISUAL CORTEX NEURONS IMPROVES SIGNAL ENCODING

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Abstract. Gamma band oscillations (25 - 70 Hz) play an important role in processing of information by neocortical neurons. In simple cells of the cat's visual cortex, it was previously shown that strength of gamma oscillations is modulated by the membrane potential oscillations at the temporal frequency of the stimulus. More recently, theoretical studies using a conductance-based neuronal model have shown that this coupling significantly improves visual stimulus encoding. Due to the availability of a broad range of genetic tools, mice had recently become an important experimental subject for research in various fields of neuroscience, including visual physiology. It has been suggested that gamma oscillations in the mouse visual cortex play a minor role in visual processing due to the lack of specialized neurons that take part in generating gamma oscillations. Here we show, using patch clamp recording from simple cells in the visual cortex of anesthetized mice, that the strength of gamma oscillations is modulated by the phase of stimulus-induced oscillations during visual stimulation with moving gratings. In addition, using patch clamp recording from mouse visual cortex neurons in slices, we demonstrated benefits of gamma activity modulation for encoding of slow sinusoidal signals into sequences of action potentials. Thus, the phenomenon of amplitude modulation of gamma oscillations by temporal frequency of stimulus, originally described in the visual system of cats, may represent a universal mechanism that improves encoding of visual information which is present even in animals with a relatively poorly developed visual system, such as mice.

Keywords: visual cortex, mice, neurons, gamma oscillation, path clamp, in vivo, action potential, signal encoding.

List of Abbreviations
RF – Receptive field
FFT – Fast Fourier transform

Introduction

Mice had recently become an important experimental subject for research in various fields of neuroscience, including visual physiology, due to the availability of a broad range of genetic tools and development of behavioral tools for testing visual function psychophysically (Carandini & Churchland, 2013). While visual acuity in mice is about 100 times lower than in primates, visual neurophysiology in mice capitalize on large scope of prior research in cats and primates. Indeed, visual cortex neurons in mice have receptive fields with overall spatial structure, orientation and directional tuning similar to that of cats and primates (Huberman & Niell, 2011). Gamma band oscillations (25–70 Hz) play an important role in different aspects of visual processing, such as synchronization of activity of neuronal ensembles (Eckhorn *et al.*, 1988; Singer, 1999; von der Malsburg, 1999), but also improving response selectivity and stimulus encoding in neuronal spiking by enhancing transformation of membrane potential fluctuations into trains of action potentials (Volgushev et al., 2002; 2003). One mechanism facilitating encoding of visual stimuli in simple cells in cat visual cortex is modulation of the amplitude of gamma-band fluctuations by the membrane potential oscillations at the temporal frequency of the stimulus, so that the gamma-band fluctuations are stronger at depolarization peaks, but weaker in hyperpolarizing phases of the stimulus frequency oscillation. Indeed, computer simulations using a conductance-based model have shown that such coupling significantly improves visual stimulus encoding (Hoch *et al.*, 2011). Here we asked whether a similar mechanism operates in mouse visual cortex. Using in vivo whole cell recording from visual cortex neurons in anesthetized mice, we tested whether visual stimuli induce high frequency, gamma-band fluctuations and whether

their amplitude is modulated by the slow, stimulus-frequency oscillations of the membrane potential. In slices from mouse visual cortex, we tested whether encoding of low-frequency sine-wave current signals injected into the cells is improved by modulation of the amplitude of gamma band fluctuations by the sine-wave phase, as observed in vivo.

Methods

All experimental procedures were in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health and were approved by the Ethical Committee of the Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences.

Animal procedures in in vivo experiments. Experiments were performed on adult (1.5–4 month) C57/Bl6 mice of both sexes. For in vivo experiments mice were anesthetized with urethane (0.7–1 g/kg body weight) intraperitoneally, a metal fixator was attached to the exposed skull and a small 200–300 mkm craniotomy above the primary visual cortex (4.5 mm posterior, 2.5 mm lateral to the Bregma) was performed under local lidocaine anesthesia. After one hour of recovery from the surgery, the mouse was placed in experimental setup. During the whole recording session typically lasting 3-4 hours, urethane anesthesia was maintained.

In vivo recording. Whole-cell recordings from visual cortex neurons made using patch pipettes filled with a solution containing (in mM): 140 K-Gluconate, 20 KCl, 4 Mg-ATP, 0.3 Na2GTP, 10 Na-Phosphocreatine, 10 HEPES, pH 7.25 (all from Sigma, USA). When filled with the solution, the electrodes had resistances of 3–4 MΩ. Membrane potential was recorded using Multiclamp 700B amplifier, digitized at 20 kHz using Digidata 1550 DAQ and pCLAMP software (Molecular devices, USA) and stored for off-line analysis.

Visual stimulation. For visual stimulation, moving sine-wave gratings were generated using Psychopy3 and presented on a monitor placed 25 cm from the mouse eye, covering 60–70 degrees of visual field. Gratings had spatial frequency of 0.04 cpd, and were moving at temporal frequency of 2 Hz. Gratings moving in 8 different directions were presented in pseudo random order, for 2 s each, separated by presentation of gray blank screen for a 0.5 s between trials.

Preparation of acute brain slices. Acute brain slices were prepared using conventional techniques. Briefly, 1–1.5-month-old mice both sexes bred in the institute's colony were deeply anesthetized with isoflurane and decapitated. The brain was rapidly removed and immersed in ice-cold oxygenated sucrose-based solution, containing, in mM: 83 NaCl, 25 NaHCO3, 2.7 KCl, 1 NaH2PO4, 0.5 CaCl2, 3.3 MgCl2, 20 glucose, 71 sucrose, bubbled with 95% O2/5% CO2. The oxygenated sucrose-based solution was used during preparation of slices and in the slice incubator. Transverse slices of the visual cortex (350 μm) were cut using a vibratome (VT1200S, Leica, Germany). After preparation slices were first incubated for 45-60 min for recovery, and then slice incubation chamber was moved to room temperature. For recording, individual slices were transferred into a recording chamber mounted on an Olympus BX-50WI microscope equipped with IR-DIC optics. ACSF solution for recording contained, in mM: 125 NaCl, 25 NaHCO3, 25 glucose, 3 KCl, 1.25 NaH2PO4, 2 CaCl2, 1 MgCl2, bubbled with 95% O2/5% CO2, pH 7.4. Recordings were made at 30–32 °C.

Recording in slices. The patch electrodes were filled with a potassium gluconate-based solution (130 mM potassium gluconate, 20 mM KCl, 4 mM Mg-ATP, 0.3 mM Na2-GTP, 10 mM sodium phosphocreatine, 10 mM Hepes) and had a resistance of 4–6 MΩ. Recordings were made with a MultiClamp 700B amplifier (Molecular Devices, USA) in the current clamp or voltage clamp mode. After amplification and low-pass filtering at 10 kHz, the data was digitized at 10 kHz and fed into a computer using Digidata 1500 interface and pCLAMP software (both from Molecular Devices, USA).

Data analysis. Using FFT filter, two components were extracted from the recorded membrane potential responses to moving gratings. The low frequency component (1.5–2 Hz) contained the stimulus frequency (2 Hz), and the high frequency component contained oscillations in the gamma-range (25–70 Hz). For estimation of the amplitude of gamma-band oscillations, rolling RMS with 100 ms window was used. This signal was used to calculate Pearson correlation with the low-frequency response component.

Orientation tuning was then calculated for the low and the high frequency components using integral of the power spectrum in the respective range (1.5–2 Hz and 25–75 Hz), and for the correlation between the low and high frequency components using Pearson correlation coefficient.

Statistical analysis. One way ANOVA with Turkey post-hoc test was used for comparison of the means.

Results

To test whether in mouse visual cortex neurons visual stimuli induce high frequency, gamma-band fluctuations and whether their amplitude is modulated by the slow, stimulus-frequency oscillations of the membrane potential, we made in vivo whole cell recording from visual cortex neurons in mice under urethane anesthesia. In neurons from layers 2-5 of the visual cortex, we recorded membrane potential responses to sine-wave moving gratings of different orientations. In each of 24 recorded neurons, responses to 12 different directions of grating movement were tested. Similarly to other species, receptive fields (RFs) of neurons in mouse primary visual cortex can be classified as simple or complex. Neurons with simple receptive fields respond to optimally-oriented moving gratings with a strong modulation of the membrane potential at temporal frequency of the stimulus (Fig. 1, a1). Action potentials are generated at depolarizing peaks of membrane potential fluctuations, and thus spike response is also strongly modulated at stimulation frequency. Out of 24 recorded neurons, 16 showed strong modulation of membrane potential and spike responses at temporal frequency of stimulation. These neurons were classified as simple and used for the purposes of the present study. The remaining 8 cells did not show significant modulation of membrane potential and spike responses at temporal frequency of stimulation; were classified as complex and excluded from the further analysis.

Figure 1, a1 illustrates typical response of a simple cell to moving gratings of optimal orientation, with clear stimulus-frequency modulation of membrane potential and spike responses. In line with modulation of membrane potential at temporal frequency of the moving grating, visual stimulation also induced high-frequency fluctuations of the membrane potential. To disentangle membrane potential response at stimulus-frequency (1.5–2 Hz) and the gamma-band component (25–70 Hz), we used fast Fourier transformation (FFT) and filtering (Fig. 1, a2, a3). This analysis also revealed a clear dependence of the amplitude of gamma-band fluctuations on the phase of stimulus-frequency membrane potential response. To quantify this relation we calculated gamma-band power in a 100 ms running window. Superposition of the stimulus-frequency oscillation of the membrane potential and the gamma-band power revealed a clear phase-locking of the two response components, so that the gamma-band fluctuations are stronger at depolarization peaks, but weaker in hyperpolarizing phases of the stimulus frequency oscillation (Fig. 1, a4).

During responses to non-optimal orientation, the cell did not show any appreciable modulation of membrane potential at stimulus frequency, and no substantial fluctuations in the gamma band (Fig. 1, b). Stimuli of other tested orientations induced membrane potential responses of intermediate magnitudes, both at the temporal frequency of gratin stimuli, as well as in the gamma-band. Both the low- and the high-frequency component of the membrane potential response had similar orientation tuning, calculated using integral power in the respective range of frequencies (Fig. 1c). Also spike responses were tuned to the same range of orien-

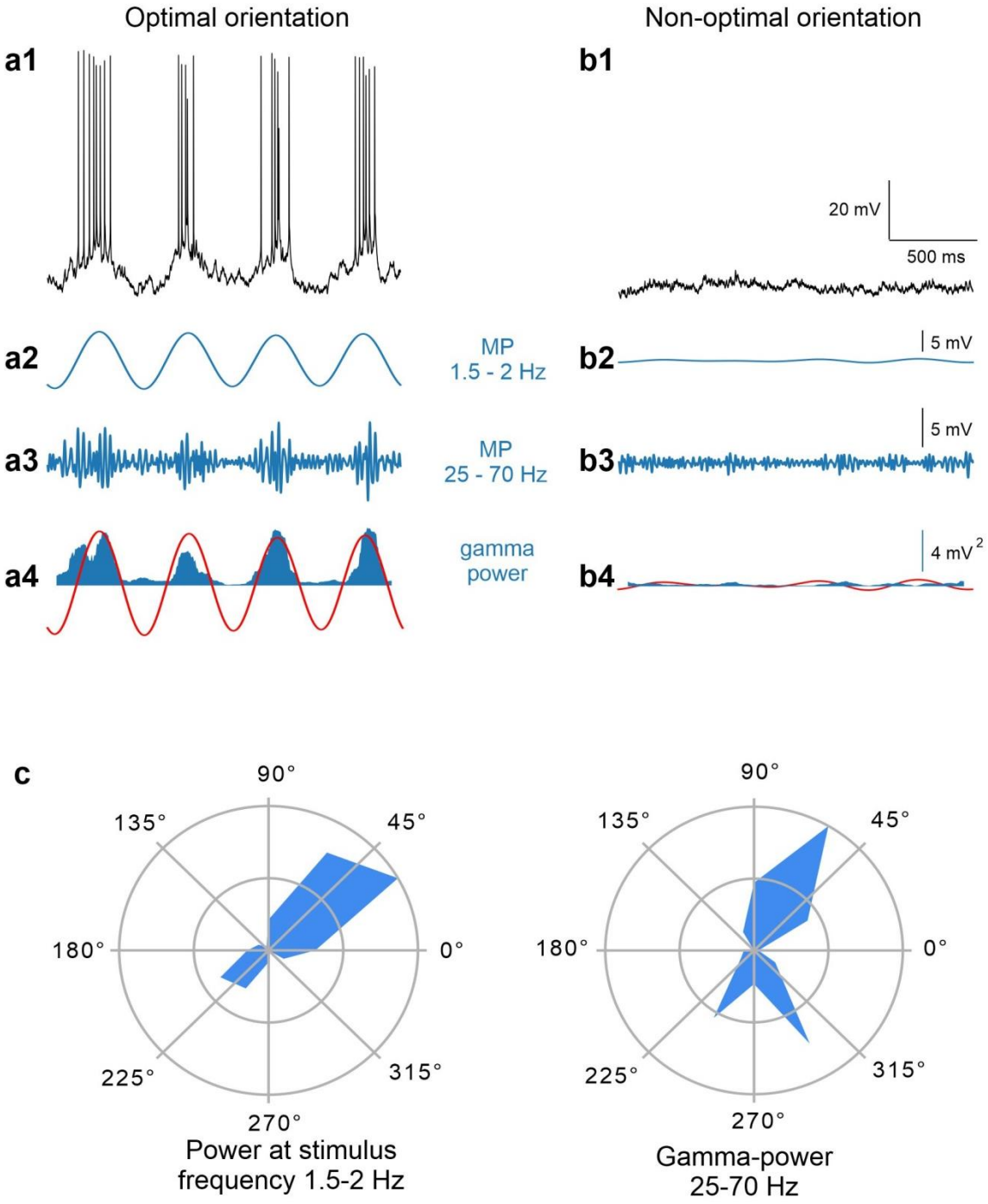

Fig. 1. In vivo, the amplitude of gamma-frequency oscillations depends on the phase of low-frequency modulation of the membrane potential induced by visual stimulation.
a, b: Membrane potential responses in example cell from mouse visual cortex to moving gratings of optimal (a) and non-optimal orientation (b). From top to the bottom: Membrane potential traces (a1, 1); Low-frequency component of membrane potential response at temporal frequency of stimulation (2 Hz, a2, b2); Gamma-band component of membrane potential response (25-70 Hz, a3, b3); Superposition of the low frequency response component and RMS gamma-power calculated in a 100-ms running window; **c**: Polar plots of direction tuning of integral power at temporal frequency of visual stimulation (1.5–2 Hz, left) and in the gamma-band (25–70 Hz, right).

tations as the membrane potential responses (data not shown).

Results, qualitatively similar to those described for the sample neuron in Figure 1, were observed in all 16 recorded neurons with simple RFs.

Thus, our in vivo experiments show that in mouse visual cortex neurons with simple RFs, visual stimuli in the optimal range induce strong fluctuations of membrane potential in the gamma-frequency range, and that the magnitude of gamma-band fluctuations is modulated by the slow, stimulus-frequency oscillation of the membrane potential.

To test whether such modulation improves encoding of low-frequency membrane potential signals into trains of action potentials, we next made recordings in slices from mouse visual cortex. We studied membrane potential and spike responses of neurons to injected currents consisting of two components, mimicking the components of membrane potential responses recorded in vivo. Low frequency component in the injected current was a 2 Hz sine wave. High frequency component was represented by different realizations of gamma-band fluctuations. Currents for injection were synthetized as repeated pattern of three cycles of a 2 Hz sine-wave. In each triad, the first cycle consisted of the sine-wave alone without any high-frequency component, in the second cycle fluctuating gamma-band component with the amplitude independent of the sine-wave phase was added, and in the third cycle gamma-band component with the amplitude modulated by the sine-wave phase was added (Fig. 2a, lower trace). Current consisting of 150 of such triads, each with individual realization of the fluctuating gamma-band component, was used in each experiment. The amplitude of injected current was adjusted to produce fluctuations of the membrane potential of the same range as observed in in vivo experiments, and an additional de- or hyper-polarizing constant current was added so that the neuron generated several action potentials during each depolarizing phase of the 2 Hz sine-wave (Fig. 2a, upper trace).

Current injection experiments were made in the layer 2/3 pyramidal neurons in slices from mouse visual cortex. In each neuron we calculated averaged number of spikes in bursts evoked by sine-wave cycles without gamma-fluctuations, with unmodulated gamma-fluctuations and with modulated gamma-fluctuations. For the sine-wave only and unmodulated gamma-band conditions, averaged number of spikes per cycle did not differ ($0{,}79 \pm 0{,}06$ and $0{,}95 \pm 0{,}01$, $P > 0{,}05$). With modulated gamma, significantly more spikes per cycle were generated compared to sine-wave only and unmodulated gamma conditions ($1{,}26 \pm 0{,}07$) (Fig. 2b, right plot). We next calculated firing rate in bursts of evoked action potentials as an inverse of the mean interspike interval in the burst. As with the number of spikes, firing rate was not different between sine-wave only and unmodulated gamma conditions, but was significantly higher in the modulated gamma condition ($0{,}94 \pm 0{,}02$; $0{,}94 \pm 0{,}02$ and $1{,}13 \pm 0{,}04$ respectively) (Fig. 2b, right plot).

Thus, results of current injection experiments in slices show that modulation of the gamma-band membrane potential fluctuations by the phase of stimulus-frequency oscillations increases both the number of spikes in bursts and their frequency within the bursts, and thus improves encoding of the stimulus into sequences of action potentials.

Discussion

Using patch clamp recording from simple cells in the visual cortex of anesthetized mice we showed, that the strength of gamma oscillations is modulated by the phase of fluctuation of the membrane potential at temporal frequency of stimulation with moving gratings. Amplitude of gamma-band oscillations was higher at depolarizing peaks, and lower at hyperpolarizing phases of the stimulus-frequency membrane potential fluctuations. Using patch clamp recording from mouse visual cortex neurons in slices, we demonstrated benefits of such modulation of gamma activity for encoding of slow sinusoidal signals into sequences of action potentials. Intracellular injection of current stimulus, representing three different types of input (stimulus alone, unmodulated gamma, and modulated gamma) showed that current

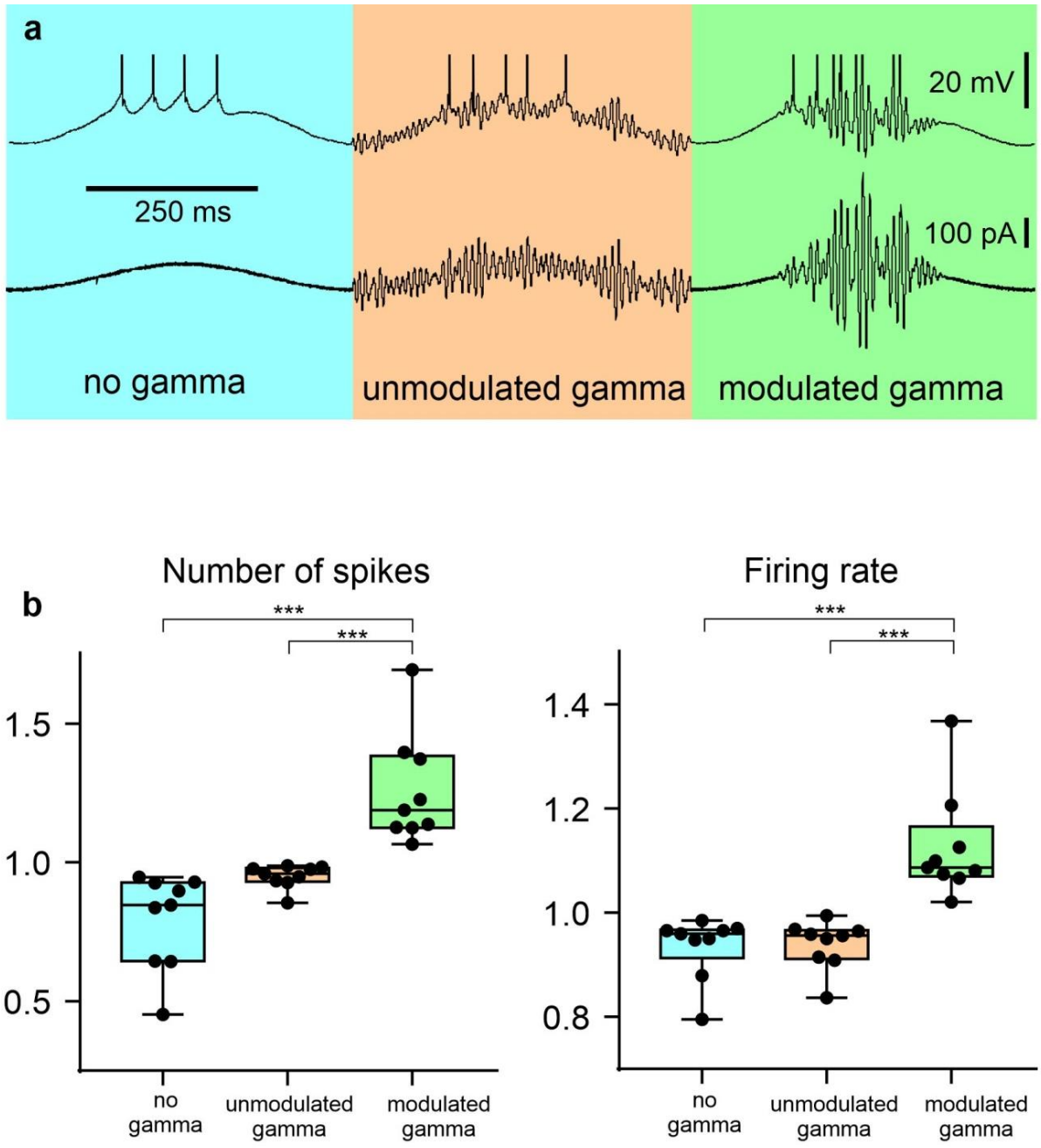

Fig. 2. In visual cortex neurons in slices, spike responses to injection of fluctuating current are enhanced by modulation of the gamma-band component of injected current by the phase of low-frequency component. **a**: Membrane potential responses of an example neuron from visual cortex (upper trace) to injection of sine-wave current (2 Hz, lower trace) without high-frequency component (first cycle, blue background); with added fluctuation gamma-band (25-70 Hz) component with amplitude independent of the phase of the sine wave (second cycle, peach background) and with gamma-component with amplitude modulated by the sine-wave phase (third cycle, green background); **b**: Mean number of spikes (left) and firing rate (right) in response to injection of sine wave current as in experiments illustrated in (a), with no gamma component, unmodulated gamma-component and modulated gamma-component; color code as in a. Top and bottom edges of the box indicate the 75th and 25th percentiles, respectively. Top and bottom whiskers indicate maximal and minimal values correspondingly (no outlier excluded). All data points are shown. Thick black horizontal line denotes the median. *** – $P < 0{,}001$, One way ANOVA with Tukey post-hoc test)

configuration mimicking in vivo recordings, with the gamma-activity modulated by the phase of slow depolarizations, induced stronger spike responses, with higher frequency and more numerous action potentials.

The important role of gamma oscillations in operation of visual cortex neurons has been demonstrated in traditional subjects for visual physiology, cats and monkeys, and in theoretical studies. Gamma-band oscillations of the membrane potential reduce the threshold for the generation of action potentials (Azouz & Gray, 2003; 2008) and make the generation of action potentials more reliable (Mainen & Sejnowski, 1995; Nowak, 1997; Volgushev *et al.*, 1998, 2003; Salinas & Sejnowski, 2000). Theoretical studies also demonstrated that modulation of the gamma-power by the phase of the low-frequency membrane potential changes improves stimulus encoding and provides higher information transmission efficiency (Hoch *et al.*, 2011). Results of our present study in mice neurons are in line with previously reported results from neurons recorded in cat visual cortex in vivo (Volgushev *et al.*, 2003; Hoch *et al.*, 2011). Interestingly, recent work in awake mice demonstrated an important role of gamma oscillations in visual cortex for visual perception (Arroyo *et al.*, 2018).

Thus, gamma-band oscillations might be universally employed in visual processing in diverse species, and the phenomenon of amplitude modulation of gamma-band oscillations by temporal frequency of the stimulus, may represent a universal mechanism that improves encoding of visual information in visual system of different species, including animals with a relatively poorly developed visual system, such as mice.

Acknowledgements

The study was supported by Russian Science Foundation, grant # 20-15-00398.

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