OPTICAL-ELECTRICAL MODULATION OF CORTICAL NEURONAL ACTIVITIES IN THE PERFUSED TISSUE SLICE

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We here propose a new experimental technique to modulate cortical neuronal activities by a combination of laser light irradiation and electrical microstimulation in the tissue slice perfused with artificial cerebro-spinal fluid (aCSF). This technique enables one to both excite and inhibit the neuron populations in a spatially confined manner.

Previously, it has been demonstrated that the infrared (IR) laser light irradiation can induce suprathreshold membrane depolarizations in invertebrate, mammalian and expression cells[1][2][3]. And also, a few studies have shown that its inhibitory effect on spike propagation in peripheral nerve bundles[4][5]. In the cerebral cortex in vivo, on the other hand, IR laser light irradiation could result in both excitations and inhibitions of neurons and glial cells, but was hard to selectively induce either one of such excitations and inhibitions[6][7][8][9]. Our method differs from those of the previous studies in technical strategy of controlling cortical neural activities. Namely, we utilized a laser light of rather shorter wavelength than the infrared one used previously, so that this near-infrared (NIR) laser irradiation preferentially induced neuronal inhibitions. And in a complimentary way, electrical microstimulation was employed to induce neuronal excitations. Also, our first application target of this technique was the cerebral tissue slice for avoiding accumulation of excessive heat by continuously perfusing the slice with a temperature-controlled aCSF.

Spatio-temporal neuronal activities in the mouse cerebral tissue slice (~0.3 mm thick; from C57BL/6J mice, P23-56) were visualized by epifluorescence imaging with Ca^{2+}-sensitive dye (Oregon Green 488 BAPTA-1-AM)[10] under an upright microscope attached with a emCCD camera (200 fps), as illustrated in Figure 1. The light wavelengths of excitation and emission of the dye were 475±20 nm and 535±22.5 nm, respectively. And a spot region in the slice (~140-μm diameter; circle in the bottom of Fig. 2A) was irradiated by the NIR laser light (~808±5-nm wavelength) from the bottom via a cover glass. The single microstimulation pulse (cathodic-first biphasic current; 10-80 μA/phase and 0.2 msec/phase) was delivered through a glass microelectrode (~6-μm tip) inserted in the slice (middle panel of Fig. 2A). Figure 2B-C shows an example of the experimental results. In the control condition (i.e. w/o the laser irradiation; Fig. 2B), the cytoplasmic Ca^{2+} rise due to the suprathreshold depolarization was induced around the stimulating electrode (arrow in Fig. 2B) at 2-7 msec after the microstimulation, and then the secondary Ca^{2+} rise indicating the post-synaptic excitation was observed in the superficial layer at 7-12 msec post-stimulus time (cf.[10]). The Ca^{2+} rise in this layer was most significant at 22-32 msec post-stimulus time. As shown in Fig. 2C, when the spot region in this layer was irradiated by the laser light, from 0.2 sec prior to the microstimulation, the Ca^{2+} rise in the spot region was significantly smaller than that in the control (e.g. compare the frames at 32 msec post-stimulus time in B and C).

The present study showed, for the first time to our knowledge, that the NIR laser light irradiation can inhibit the microstimulation-induced neuronal excitations in a spatially confined manner in the cerebral tissue slice. In the experiment, the laser light was applied to the layer II/III where the neuronal responses were induced mostly by the excitatory synaptic inputs from the layer IV [10]. Thus, it is indicated that the laser light can inhibit the physiologically relevant activities of the cortical neural circuit. We believe that the experimental technique proposed here is useful for controlling cortical neuronal activities, at least in vitro, by a non-pharmacological mean.

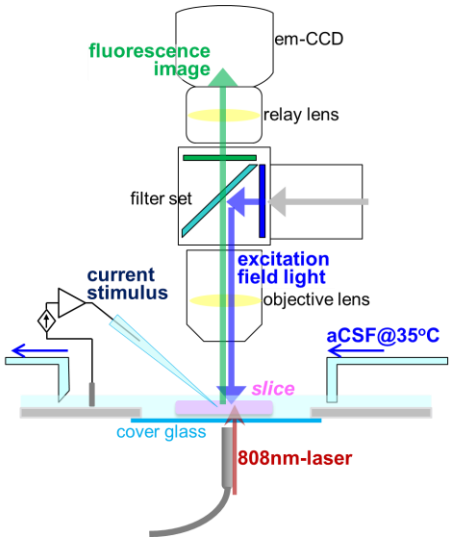

Fig.1 Schematic diagram of the experimental set-up with the laser light irradiation, the electrical microstimulation, and the epi-fluorescence imaging of the cerebral tissue slice perfused with temperature-controlled aCSF.

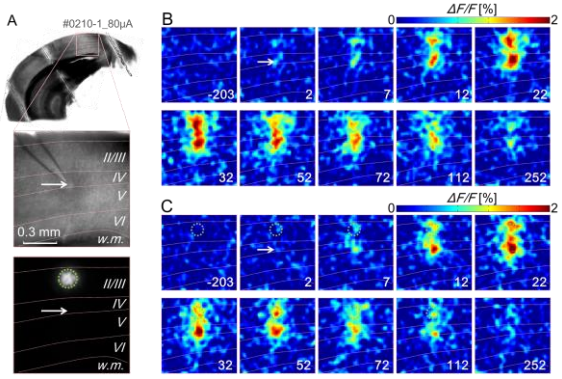

Fig. 2 The CaSD imaging of neural responses to the microstimulation to the layer IV of the primary visual cortex in a mouse cerebral tissue slice. A, The upper and middle panels show differential interference contrast images of the slice and the cortex with an expanded view, respectively. The lower panel shows the laser light spot irradiated onto the layer II/III. Circles indicate widths of the laser spot at the half maximum of the intensity. Arrow indicates the stimulating electrode tip. B-C, Pseudo-color time lapse images of the dye fluorescence change in the slice responding to the stimulus pulse without (B) or with the laser irradiation (C). The numbers indicates the post-stimulus time in msec.

REFERENCES:

[1] H. Hirase, V. Nikolenko, J.H. Goldberg, R. Yuste, "Multiphoton stimulation of neurons," J. Neurobiol., vol.51(3), pp.237-247, 2002.

[2] J. Wells, C. Kao, K. Mariappan, J. Albea, E.D. Jansen, P. Konrad, A. Mahadevan-Jansec, "Optical stimulation of neural tissue in vivo," Opt. Lett., vol. 30(5), pp.504-506, 2005.

[3] C.-P. Richter, A.I. Matic, J.D. Wells, E.D. Jansen, and J.T. Walsh, " Neural stimulation with optical radiation," Laser Photon. Rev. vol.5(1), pp.68-80, 2011.

[4] Z. Mou, I.F. Triantis, V.M. Woods, C. Toumazou, and K. Nikolic, "A simulation study of the combined thermoelectric extracellular stimulation of the sciatic nerve of the Xenopus laevis: the localized transient heat block," IEEE Trans. Biomed. Eng., vol.59(6), pp.1758-1769, 2012.

[5] A.R. Duke, M.W. Jenkins, H. Lu, J.M. McManus, H.J. Chiel, E.D. Jansen, "Transient and selective suppression of neural activity with infrared light," Sci. Rep., vol. 3, article#2600, 2013.

[6] H.-J. Feng, C. Kao, M.J. Gallagher, E.D. Jansen, A. Mahadevan-Jansen, P.E. Konrad, R.L. Macdonald. Alteration of GABAergic neurotransmission by pulsed infrared laser stimulation. J. Neurosci. Methods vol.192(1), pp.110-114, 2010.

[7] J.M. Cayce, R.M. Friedman, and E.D. Jansen, A. Mahadevan-Jansec, and A.W. Roe, "Pulsed infrared light alters neural activity in rat somatosensory cortex in vivo," NeuroImage vol.57(1), pp.155-166, 2011.

[8] J.M. Cayce, M.B. Bouchard, M.M. Chernov, B.R. Chen, L.E. Grosberg, E.D. Jansen, E.M.C. Hillman, A. Mahadevan-Jansen. Calcium imaging of infrared-stimulated activity in rodent brain. Cell Calcium, vol.55(4), pp.183-190, 2014.

[9] J.M. Cayce, R.M. Friedman, G. Chen, E.D. Jansen, A. Mahadevan-Jansen, and A.W. Roe, "Infrared neural stimulation of primary visual cortex in non-human primates, " NeuroImage, vol.84, pp.181-190, 2014.

[10] M. Osanai, S. Tanaka, Y. Takeno, S. Takimoto, and T. Yagi, "Spatiotemporal properties of the action potential propagation in the mouse visual cortical slice analyzed by calcium imaging," PLoS ONE, vol.5(10), article#e13738, 2010.