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Auditory Cortical Plasticity Induced by Intracortical Microstimulation under Pharmacological Blockage of Inhibitory Synapses

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Abstract— **Electrical stimulation that can reorganize our neural system has a potential for promising neurorehabilitation. We previously demonstrated that temporally controlled intracortical microstimulation (ICMS) could induce the spike time-dependant plasticity and modify tuning properties of cortical neurons as desired. A 'pairing' ICMS following tone-induced excitatory post-synaptic potentials (EPSPs) produced potentiation in response to the paired tones, while an 'anti-pairing' ICMS preceding the tone-induced EPSPs resulted in depression. However, the conventional ICMS affected both excitatory and inhibitory synapses, and thereby could not quantify net excitatory synaptic effects. In the present work, we evaluated the ICMS effects under a pharmacological blockage of inhibitory inputs. The pharmacological blockage enhanced the ICMS effects, suggesting that inhibitory inputs determine a plastic degree of the neural system. Alternatively, the conventional ICMS had an inadequate timing to control excitatory synaptic inputs, because inhibitory synapse determined the latency of total neural inputs.**

I. Introduction

Neurorehabilitation will potentially benefit from electrical stimulation that can modify synaptic strength as desired. Previous studies demonstrated that temporally controlled intracortical microstimulation (ICMS) could reorganize tuning properties of neurons in the cerebral cortex [1], [2]. In these experiments, the timings of ICMS were based on the spike time-dependent plasticity rule, by which single postsynaptic action potentials (APs) following excitatory post-synaptic potentials (EPSPs) strengthen the synapse, while APs preceding EPSPs weaken the synapse [3]. Therefore, ICMS generating APs before external-stimuli-induced EPSPs, called "anti-pairing ICMS," can weaken the synapse, whereas ICMS following EPSPs, or "pairing ICMS," can strengthen the synapse. In these studies, however, ICMS might affect both excitatory and inhibitory synapses, and thereby could not quantify the net excitatory synaptic effects.

In the present work, we attempt to evaluate net excitatory synaptic effects of ICMS under a pharmacological blockage of inhibitory inputs. We first administer antagonist of inhibitory synaptic receptors ($GABA_A$), and measure temporal changes of tone-induced neural responses in the auditory cortex of a rat. Second, we measure ICMS-induced changes of tuning properties of auditory cortical neurons with and without the antagonist. On the basis of these experimental results, we infer the characteristics of excitatory synaptic plasticity.

II. Material and Methods

A. Animal Preparation

Wistar rats weighing 200 – 300 g were anesthetized with isoflurane (1 – 1.5 %), and fixed to a stereotaxic holder. The temporal skull and dura mater were partly removed to expose

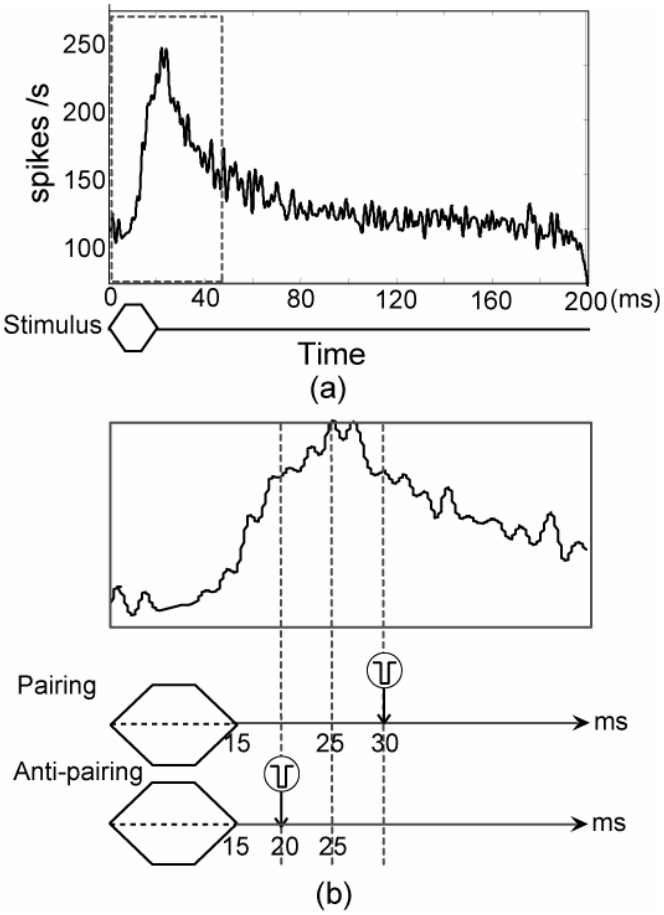

Fig. 1. Experimental paradigm. (a) PSTH of tone-evoked neural responses. (b) The timings of pairing and anti-pairing ICMS. These timings were determined on the basis of PSTH.

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the auditory cortex, and a hole was drilled at the vertex in order to insert a reference electrode. The reference electrode was placed to make an electrical contact with dura mater and fixed to the skull with dental cement. A subcutaneous needle electrode was inserted around the neck serving as a ground electrode.

Finally, we penetrated tungsten microelectrodes into the cortex, and advanced at a depth of approximately 500 μm from the cortical surface. Each electrode has a 30μm diameter.

B. Experimental procedure

We measured tone-induced neural activities in the auditory cortex and determined the timings of paring and anti-pairing ICMS on the basis of the post-stimulus latency of the neural activities. We then compared pre- and post-ICMS tuning properties of tone-induced neural responses. Similarly, we examined the ICMS effects under an administration of bicuculline-methiodide (BMI; 10 μM), antagonist of $GABA_A$ receptor [4].

Neural signals were amplified with a gain of 5,000 and a digital filter with a passband of 0.75 – 7.5 kHz (Cyberkinetics, Inc., Cerebus Data Acquisition System). Tone bursts at 30 – 90 dB SPL (Sound Pressure Level; re, 20μPa) and with frequencies of 5 – 50 kHz served as test stimuli. The test tones had a rise and fall time of 5 ms and a duration of 15 ms. The test tones were delivered from a speaker 10 cm in front of a rat's ear, contralateral to the exposed cortex. We obtained post-stimulus time histograms (PSTHs) of tone-induced neural activities, and explored the mean latency of onset response. The timings of pairing and anti-paring ICMS were set at 5 ms after and before the mean latency, respectively (Fig. 1). ICMS were presented for an hour at 1.7 Hz in conjunction with acoustic tones with a particular frequency. Tones with other frequencies were also given without ICMS in order to avoid habituation. Each ICMS was a bipolar, biphasic, charge-balanced and constant current pulse. The current and duration per phase were 20 μA and 100 μs, respectively.

We investigated pre- and post-ICMS frequency tuning properties evoked by 50 – 65 dB SPL tones, and quantified ICMS-induced changes as follows: 20 $\log_{10}$ (response magnitude at pre-ICMS frequency / response magnitude at post-ICMS frequency). Response magnitude was defined as a proportion of a number of spike potentials (SPs) at a particular test frequency to a number of SPs at all frequencies.

We also examined how long ICMS effects lasted, by measuring tuning properties of tone-induced neural responses every 10 minutes after a cessation of ICMS.

All the experiments were performed in accordance with the guidelines of "Animal Experiments Committee of the University of Tokyo".

III. RESULTS

Fig. 2 shows PSTHs of tone-evoked responses with and without BMI. The vertical axis represents the average of discharge rate in response to all the test tones. Administration of BMI delayed the mean latency by a few ms and blunted the attenuation after the mean latency. We also measured the difference between the two conditions and estimated the mean latency of inhibitory inputs. The average peak of the difference was 2.4 ms later than the peak of PSTH with BMI, implying that inhibitory effects peaked in a few ms after excitatory effects.

Fig. 3 shows frequency tuning properties of pre- and post-administration of BMI. The gray level in the figure indicates a discharge rate of SPs within 35-ms post-stimulus latency to each tone, whose frequency and intensity are given by the

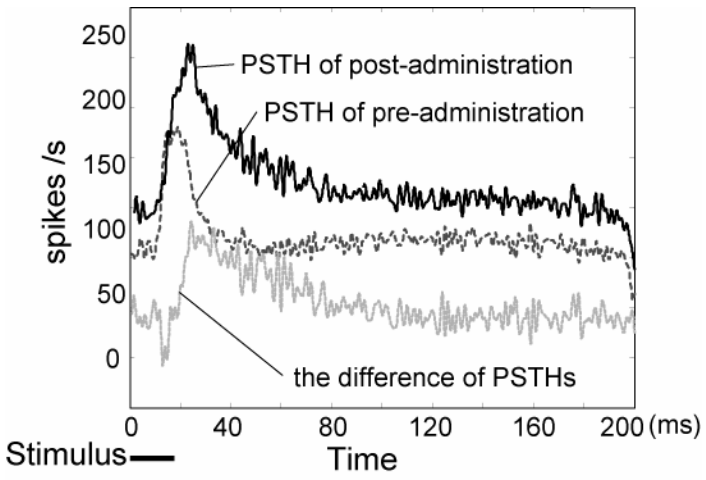

Fig. 2. PSTH of tone-evoked neural responses with and without antagonist and the difference between these PSTHs.

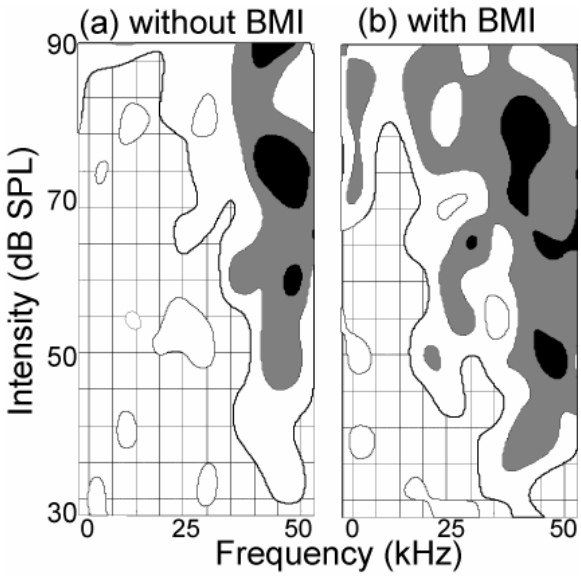

Fig. 3. Frequency tuning properties of auditory cortical responses with and without antagonist. Contour lines indicate magnitude of neural responses to corresponding test tones: white, 25 %; light shading, 50 %; and black, 75 % of maximum responses.

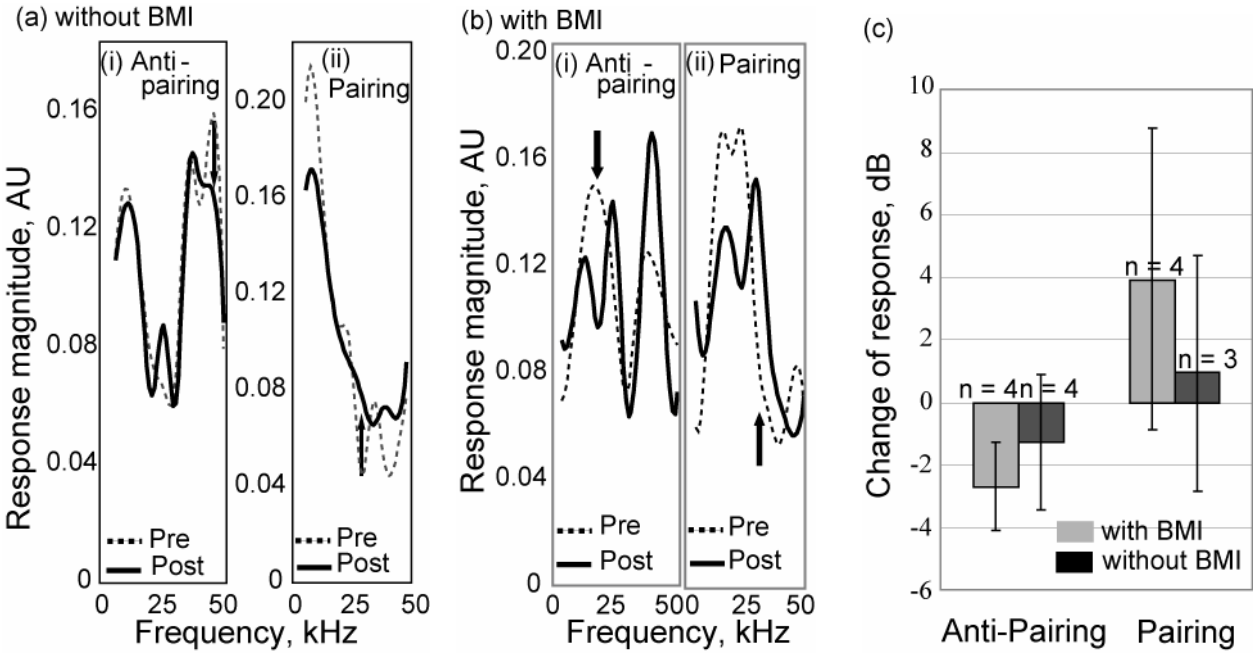

Fig. 4. Pre- and post-ICMS frequency response histogram with BMI (a) and without BMI (b): (i), anti-paring ICMS; (ii), paring ICMS. Arrows indicate frequencies of test tones associated with ICMS. (c) Effects of pairing and anti-pairing ICMS with and without BMI administration.

horizontal and vertical axes, respectively. Fig. 3 (a) indicates that neurons around the recording sites selectively responded to a 45-kHz tone, and in other words, had a characteristic frequency at 45 kHz. Fig. 3 (b) shows that BMI broadened the frequency selectivity of these neurons.

Fig. 4 shows frequency response histograms at 50 – 65 dB SPL with and without BMI in the pre- and post-ICMS recordings. Fig. 4 (a, i) indicates that neurons around the recording site were most responsive to 45-kHz tones in pre-ICMS recording. When anti-paring ICMS was administered with 45-kHz tones at this site, responses to 45-kHz tones decreased, and in turn, responses to 35 kHz tones peaked. In contrast, pairing ICMS with 30-kHz tones could enhance responses to 30 kHz tones as shown in Fig. 4 (a, ii). Figs. 4 (b) indicate that similar effects were also observed when BMI was administered.

Fig. 4 (c) compares ICMS effects with BMI to those without BMI. ICMS effects with BMI tended to be larger than those without BMI, suggesting that BMI enhanced ICMS effects.

Fig. 5 shows how anti-paring ICMS effects recovered with time, suggesting that ICMS-induced changes of tuning properties were reversible within an hour. This phenomenon was also observed for pairing ICMS effects.

IV. DISCUSSION

The present experimental results demonstrated that administration of BMI delayed the mean latency by a few ms, blunted the attenuation of discharge rates after the mean latency and broadened the selectivity of frequency. These results are consistent with previous studies in the auditory and visual cortex, suggesting that inhibitory inputs enhanced the time accuracy of the excitatory inputs and sharpened the selectivity of the neural responses [5], [6]. Thus, BMI adequately blocked the inhibitory inputs in the present experiments.

Our results suggested that BMI tended to enhance ICMS effects. There are several possible reasons for this tendency. First, ICMS timing we used without BMI was not effective to modify excitatory synapses. On the basis of the spike time-dependent plasticity rule, ICMS effects would be larger when the timing of ICMS is closer to the timing of excitatory synaptic inputs [3]. The peak of PSTH with BMI delayed 3 – 4 ms as compared to that without BMI, suggesting that the peak of PSTH without BMI did not correspond to the peak of the excitatory synaptic inputs. This stagger would decrease ICMS effects. Second, inhibitory synaptic inputs have a potential to regulate the degree of the synaptic plasticity. A previous study demonstrated that BMI increased long term potentiation (LTP) induced by the electric stimulus in hippocampal slices of rodents [7]. In addition, mice whose gene related with inhibitory synapses was knocked out did not have a critical period for formation of functional organization in the visual cortex [8]. This result implies that a blockage of inhibitory inputs would preserve the abundant plasticity of an immature brain.

The ICMS-induced changes of tuning properties were reversible with time, suggesting that neural system has independent mechanisms to induce short-term changes and to fixate the changes. Indeed, a previous study demonstrated that administration of acetylcholine is required to fixate ICMS- induced tuning properties over the long term in the bat

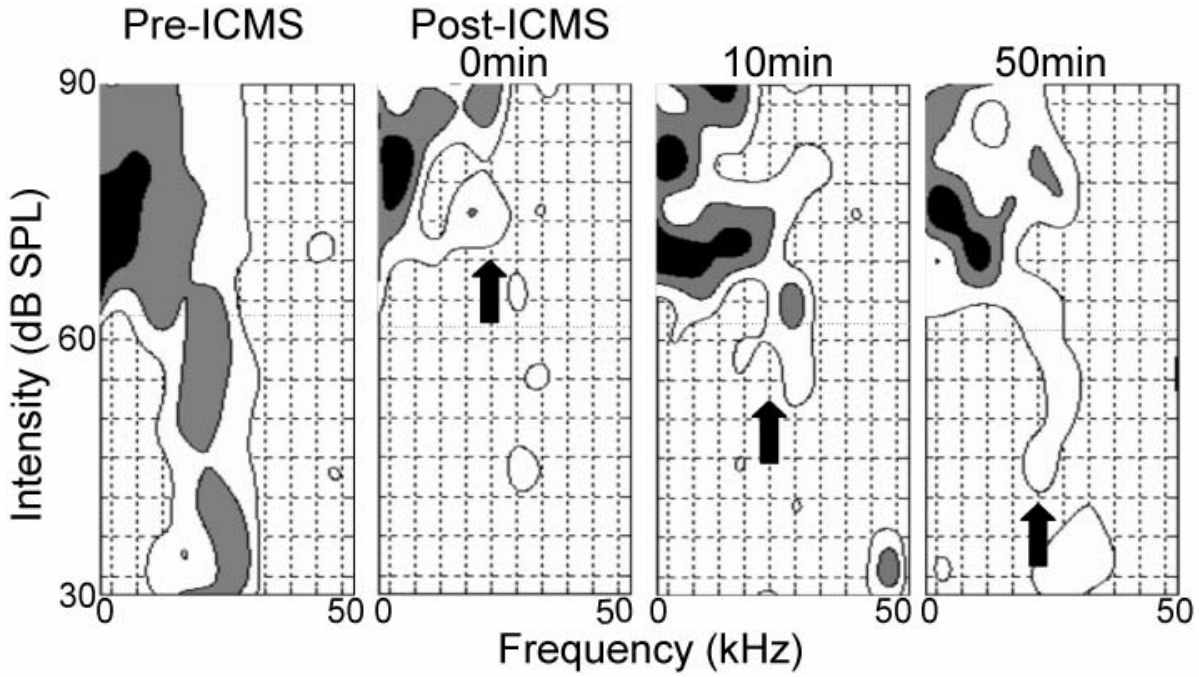

Fig. 5 Post-ICMS recovery of frequency tuning property.

auditory cortex [9]. Other study also demonstrated that learning-induced plasticity in the auditory cortex was promoted by stimulation of midbrain dopamine neurons that have cortical projection [10]. These results suggest that particular chemical substances are required to fixate plastic neural changes.

A previous study reported a spike time-dependent plasticity for inhibitory synapses at the hippocampal slices of rats [11]. Analogous to the pairing and anti-paring ICMS that could modify excitatory synaptic strength, there is a possibility that ICMS could modify inhibitory synaptic strength. The difference of PSTH with and without BMI in the present results would be helpful information to determine the timing of ICMS for modifying inhibitory synaptic inputs. The combination of excitatory and inhibitory synaptic modification by ICMS would be more powerful tool to reorganize neural systems.

REFERENCES

[1] J. Suzurikawa, K. Hisada, M. Nakao, K. Kaga, R. Kanzaki, and H. Takahashi, "Reorganization of auditory cortex by pairing and anti-pairing intracortical microstimulation," *Proceedings of 2nd International IEEE EMBS Conference on Neural Engineering*, pp.594-597, 2005.

[2] S. Schuett, T. Bonhoeffer, and M. Hubener, "Pairing-induced changes of orientation maps in cat visual cortex," *Neuron*, vol. 32, pp. 325-337, 2001.

[3] L.I. Zhang, H.W. Tao, C.E. Holt, W.A. Harris, and M. Poo, "A critical window for corporation and competition among developing retinotectal synapses," *Nature*, vol. 395, pp. 37-44, 1998.

[4] M.S. Jones, D.S. Barth, "Effects of bicuculline methiodide on fast (> 200Hz) electrical oscillations in rat somatosensory cortex," *J. Neurophysiol.*, vol.88, pp. 1016-1025, 2002.

[5] M. Wehr, A.M. Zador, "Balanced inhibition underlies tuning and sharpens spike timing in auditory cortex," *Nature*, vol. 426, pp.442-446, 2003.

[6] H. Sato, N. Katsuyama, H. Tamura, Y. Hata, and T. Tsumoto, "Mechanisms underlying orientation selectivity of neurons in the primary visual cortex of macaque," *J. Physiol.*, vol. 494, pp. 757-771, 1996.

[7] R.M. Meredith, A.M. Floyer-Lea, and O. Paulsen, "Maturation of long-term potentiation induction rules in rodent hippocampus: Role of GABAergic inhibition," *J. Neurosci.* vol.23, pp. 11142-11146, 2003.

[8] M. Fagiolini, T.K. Hensch, "Inhibitory threshold for critical period activation in primary visual cortex," *Nature*, vol. 404, pp. 183-186, 2000.

[9] X. Ma, N. Suga, "Long-term cortical plasticity evoked by electric stimulation and acetylcholine applied to the auditory cortex," *Proc. Natl. Acad. Sci. USA.,* vol. 102, pp. 9335-9340, 2005.

[10] S. Bao, V.T. Chan, M.M. Merzenich, "Cortical remodelling induced by activity of ventral tegmental dopamine neurons," *Nature*, vol. 412, pp.79-83, 2001.

[11] M.A. Woodin, K. Ganguly, and M. Poo, "Coincident Pre- and Postsynaptic activity modifies GABAergic synapses by postsynaptic changes in Cl^- transporter activity," *Neuron*, vol. 39. pp. 807-820, 2003.