

Temporal Dynamics Revealed by Paired Stimulation in the Primary Somatosensory Cortex of Anesthetized Rats

Sang-Eok Kim, Young-Sil Kim and Hyung Cheul Shin*

Department of Physiology, College of Medicine, Hallym University,
Chuncheon 200-702, Korea

ABSTRACT

We report here that temporally paired stimulation to the peripheral body of rat results in synchronized time locked gating of sensory transmission to the primary (SI) somatosensory cortex. Gating of sensory inflow at the SI cortex was related to inter-spike interval of paired stimulus, suggesting that previous sensory experience may strictly dictate subsequent sensory input. Blocking the inhibitory synapses in the SI cortex showed similar result, suggesting that lateral inhibition in cortex can not explain the cause of this sensory gating. The curve-fitted characteristic function of paired stimulation shows early near gating-off and long lasting but shallow suppression of sensory transmission. This characteristic is similar to the activity dependent sensory modulation in nerve fibers. In conclusion, we here propose active modulation in axon fiber as possible mechanism of time-locked synchronized gating, but at present, we can't fully disregard synaptic modulation.

Key words: Somatosensory, paired stimulation, temporal dynamics, sensory processing

INTRODUCTION

Previous sensory experience modulates sensory transmission to the CNS. Especially in the auditory system, it is called temporal masking. "Forward" maskers precede target in time and modulate the targeted afferent signal, and "backward" maskers follow target in time and modulate the targeted afferent signal (Weisenberger, 1994). This temporal masking modeled its property based on psychophysical studies on human subjects (Plack, 1998;

Formby, 2002; Wiegand, 1999; Takeshi et al., 1971) studied physiological mechanism of it by single-unit analysis in the cat's cochlear nucleus and inferior colliculus. They suggested that the mechanism of forward masking may be due to the post-excitatory inhibition of some slow-adapting neurons and to synaptic inhibition for fast-adapting ones. Similar psychophysical studies were widely done in the visual system of human subjects and also models were constructed without revealing its underlying physiological mechanisms (DeMarco, 2000).

Similar studies have also been carried out in the somatosensory system (Weisenberger, 1994). Studied vibro-tactile temporal masking comparing somato-

*To whom correspondence should be addressed.
TEL: 033-248-2585. FAX: 033-255-1640
e-mail: hcshin@hallym.ac.kr

sensory with auditory system. He showed how masker duration, masker intensity, and inter-stimulus interval (ISI) interact in detection masking tasks, and investigated combined masking effects.

In view of nerve fiber (axon), the history of impulse transmission can be stored as excitability changes in the nerve fibers and axonal branches. This stored information changes dynamically and may function as a non-synaptic information processing (Shin and Raymond, 1991; Shin, 1996; Waikar et al., 1996). In this study, we focused on the aftereffects of just preceding stimulus to the next afferent transmission in the somatosensory cortex using multichannel single unit recording method. Our experimental result shows a critical time period in which afferent transmission is totally blocked. We modeled its property mathematically and suggest possible mechanisms based on the characteristic function. We show additional experimental result showing that cortical GABAergic lateral inhibition can not describe this phenomenon.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats (250~300 g, n=7) were used in this study. Animals were anesthetized with i.p. injection of urethane (Sigma, USA, 20, 7 ml/kg) and transferred to a stereotaxic apparatus. After removal of the skin and soft tissue, a relatively large craniotomy (3~4 mm diameter) was made over the SI cortex responsible for left whisker pad of rat. Dura mater was removed. The vibrissae region of right somatosensory cortex was identified according to somatosensory map (Chapin and Lin, 1984), and 16-channel multi array recording electrodes (tungsten microwire, A-M systems, USA, 75 μ m diameter, teflon-coated) were positioned with the tips of electrodes perpendicular to the cortex. Then it was lowered into the layer IV of SI cortex with hydraulic micro mover (Narishige, Japan). Each 16-channel array consisted of two rows of eight micro-wires. The distance between the two rows was 200 μ m and the interval between microwire within a row was 100 μ m. Receptive field of a neuron was verified by listening to the amplified signal through an audio speaker while deflecting a whisker. Individual whiskers were stimulated at 5mm distally from their base by computer controlled

solenoid. The timing accuracy of control pulse falls within 2 μ sec. The response time of the solenoid is about 10 msec. One whisker was selected and hooked by the stimulator, and the direction of whisking was down and up with 1 msec of pulse duration. Intensive care was done not to touch adjacent whiskers.

Neuronal spikes were pre-amplified and fed into MNAP (Multichannel neuronal acquisition processor) system (32 channels, Plexon Inc, USA). The MNAP sorts neuronal spikes in real time with maximum of 4 units from a channel. Sorting parameters, such as wave shape and voltage threshold, were set by control PC (P-III, 440 MHz). A session of recording was done for 20min. Timing data for each sorted spike are stored in control PC, and used later in off-line analysis using Nex software (Nex Technology, USA).

To see the aftereffects of an immediately preceding stimulus on the responsiveness to the next coming stimulus, one epoch of stimulus is composed of two serially applied stimuli. In an epoch, the preceding stimulus is conditioning purpose, and the following is test stimulus. Each of the two stimuli is separated with specified inter-stimulus intervals (ISI, 20, 40, 60, 80, 100, 120 and 140 msec). In each stimulus, duration of deflection was 1ms, and each epoch was given with a 0.5 Hz of frequency for 15 min for each ISI. We named this stimulation protocol as paired stimulation.

As we introduced earlier, post-excitatory inhibition (PEI) of neurons can be a possible mechanism describing critical temporal gating in the cortex. The PEI is known as the result of GABAergic lateral inhibition (Sachdev, 2000). Here we tried to block GABA effect in cortex by topical application of bicuculline, GABA_A antagonist (50 μ M, FW=509.3, Sigma, USA) to the recording site. To confirm bicuculline effect, we verified afferent activity in the cortex after 30 min of application while deflecting selected whisker (Jung and Shin, 2004).

RESULTS

In this study, we recorded overall 46 single units. Three examples of single units recorded and sorted in real-time are shown in Fig. 1. In Fig. 2 except (A), the 1st peak was resulted from conditioning

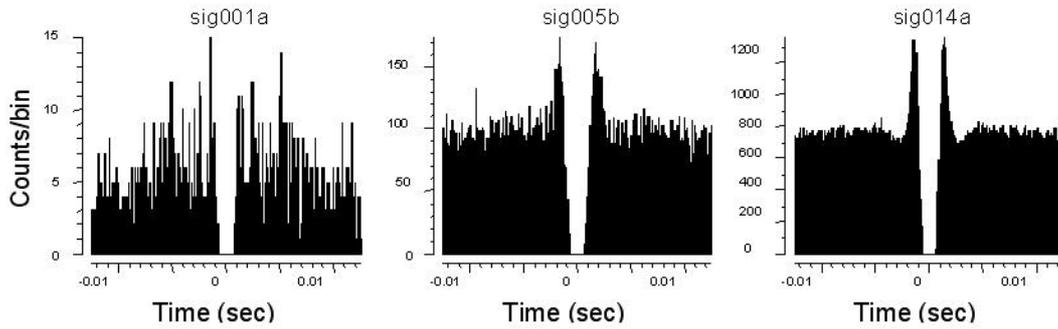


Fig. 1. Autocorrelation histogram. Examples of 3 autocorrelations from 3 single units recorded and sorted in real-time. Each graph confirms correct spike sorting (bin size=0.075 ms.).

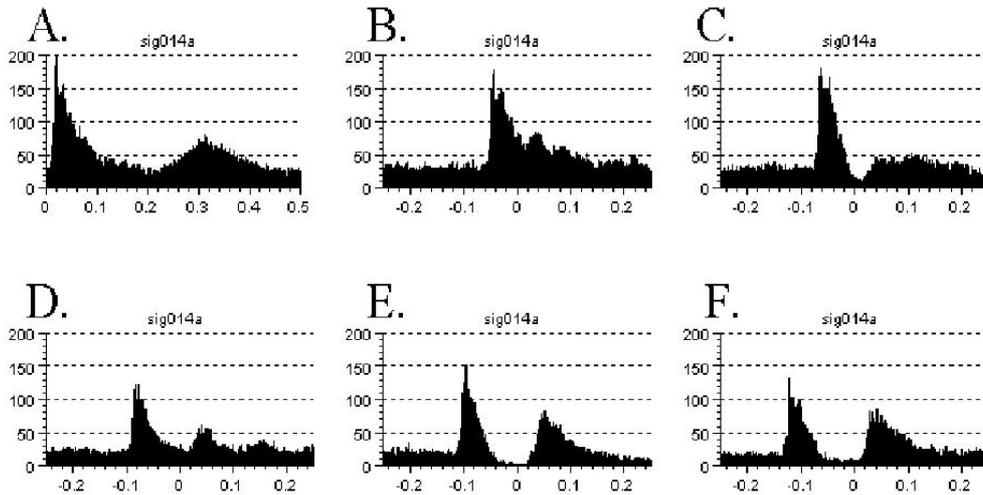


Fig. 2. Modulation of Afferent transmission in the paired stimulation protocol. Each PETH (Peri-event time histogram) shows C2 whisker activity spikes. Testing stimuli were given at time $t=0$. A) Testing stimuli only, B-F) at different ISI (ISI=40, 60, 80, 100 and 120 ms).

stimuli, and the 2nd from testing stimuli. In (A), we can see E1 peak around 15 ms post stimulus and E2 peak around 300 ms, and it shows no prominent post excitatory inhibition (PEI) period. In (B), we can see that much of activities from testing stimulus were decreased but it is still spotted around 15 ms post stimulus. But in (C), we can't observe the position of 2nd response, but we see prominent PEI instead, and it is located just around 15 ms post-stimulus. From (D) to (F), we can notice the reappearance of 2nd response as ISI widens.

Each neuron's activity is dependent on ISI (Fig. 3). We can observe strict afferent blocking (94.3%) around 60 msec post-stimulus in every neurons without exception. The bar graph in right panel of

averaged percent change can be curve fitted with following exponential expression.

$$\text{Activity \%change} = -3.515x e^{-0.0175x} \quad (1)$$

(x: ISI in msec, ≥ 0)

Jung and Shin (2004) presented systematic experimental result that shows the inhibitory effect of GABA in view of afferent sensory transmission to the SI cortex of anesthetized rats. After application of bicuculline, afferent sensory transmission to SI cortex has significantly increased maximum over 120% by 50 min and then decreased until 40% by 90 min. The down sloping shows naturally decreasing pattern. Moreover, the application of bicuculline also exhibited newly firing neurons, which were

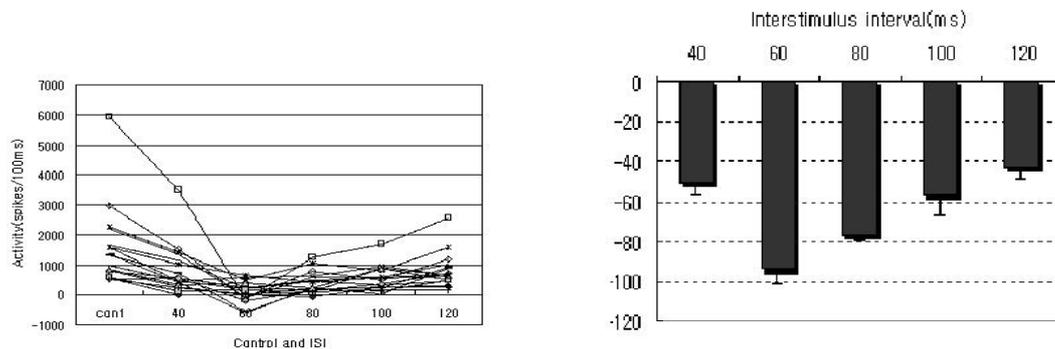


Fig. 3. Modulation of afferent transmission in each ISI. (Left) Most active unit (blank rectangle) is supposed to be the principal neuron of C2 whisker. All of them shows strict blocking in 60ms of ISI (n=17) (Right). Averaged percent changes in afferent transmission depending on ISI (ISI=40, 60, 80, 100 and 120 ms).

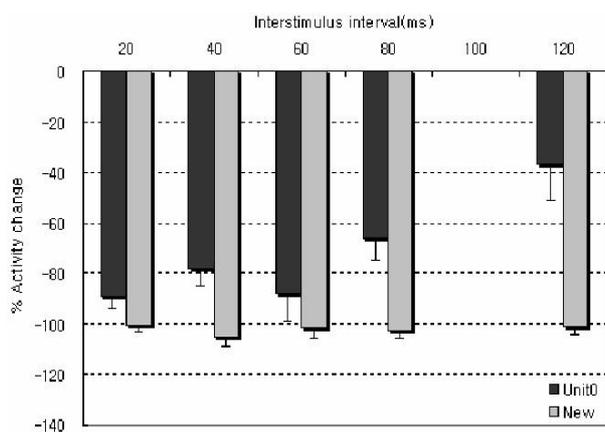


Fig. 4. Modulation of afferent transmission during bicuculline application. Black bar, labeled Unit0, represents originally firing neurons (n=9), and gray bar, labeled New, represents newly firing neurons (n=4).

silent before application, as reported previously by (Jung and Shin, 2004).

As in Fig. 4, temporal gating in each ISI for originally active neurons still exists during bicuculline application. But here we can observe a few differences compared to the temporal gating without bicuculline. The effect of ISI which shorter than 60 msec on critical gating is not prominent here. It is likely that the amount of gating before 60 msec is larger than that of without bicuculline state. Recovery of afferent transmission after 60 msec is similar to without bicuculline state.

Interestingly, newly firing neurons show near total block of afferent transmission in every ISI without

exception.

DISCUSSION

The aftereffects of impulse activity on the threshold for electrical stimulation or on the conduction velocity (CV) have been termed as 'activity dependence'. Both threshold and conduction velocity were changed after application of conditioning pulses (CPs) in single axon fibers, regardless of central or peripheral origin. In mammals, temporal changes of activity dependent membrane excitability are composed of 4 different phases. Those are refractory, super-excitable, hypo-excitable-1 (H1) and hypo-excitable-2 (H2) phases (Shin and Raymond, 1991; Shin, 1996). The 60 msec blocking of afferent transmission seen here in rat trigeminal somatosensory system is similar to the 'H1' phase. The long lasting raised threshold, 'H2' phase could be predicted from the exponential equation (1). The synchronized gating out among many neurons also suggests either subcortical or peripheral axonal mechanisms.

But here, we can not fully disregard the effect of subcortical modulations, because our experiment was not done with isolated single fiber. We could rule out GABAergic lateral inhibition in the SI cortex as the cause of temporal gating. The time period of inhibition, 60 msec, well coincides with the PEI (post excitatory inhibition) period, which is known as the result of GABAergic inhibition (Sachdev et al., 2000). But our experiment with bicuculline onto

the cortex still revealed similar result.

A phenomenon known as forward temporal masking in auditory system is very similar to our result except the beginning of masking (Watanabe, 1971). Their result shows a gradual decrease in masking from $t=0$ in both cochlear nuclear neurons and inferior colliculus neurons. In this study, we have critical time period around 60 msec, and after that we have exponential decrease in masking. Takeshi and Zyunichi explain the mechanism with PEI of some slow-adapting neurons and synaptic inhibition for fast-adapting ones. But they did not present any experimental evidences yet.

In conclusion, we demonstrated temporal gating of sensory transmission in the somatosensory system of rats using multiple single unit recording. Topical application of bicuculline onto the recording site suggests that lateral inhibition by inhibitory interneuron be not involved in this afferent gating. From the characteristic dependence of temporal gating on ISI, we suggest activity dependent modulation as a possible mechanism of temporal sensory gating. The characteristic function can be utilized in the development of vibro-tactile communication device.

ACKNOWLEDGEMENT

This work was supported by the '03 Neurobiology Research Program from the Korea Ministry of Science and Technology.

REFERENCES

Chapin JK and Lin CS (1984) Mapping the body represen-

- tation in the SI cortex of anesthetized and awake rats. *J Comp Neurol* 229:199-213.
- DeMarco PJ, Hughes A and Purkiss TJ (2000) Increment and decrement detection on temporally modulated fields. *Vision Res* 40:1907-1919.
- Formby C, Rutledge JC and Sherlock LP (2002) Exponential processes in human auditory excitation and adaptation. *Hearing Res* 3813:1-16.
- Jung SC and Shin HC (2002) Suppression of temporary deafferentation-induced plasticity in the primary somatosensory cortex of rats by GABA antagonist. *Neurosci Lett* 334:87-90.
- Plack CJ and Oxenham AJ (1998) Basilar-membrane non-linearity and the growth of forward masking. *J Acous Soc Am* 103:1598-1608.
- Sachdev RN, Sellien H and Ebner FF (2000) Direct inhibition evoked by whisker stimulation in somatic sensory(SI) barrel field cortex of the awake rat. *J Neurophysiol* 84: 1497-1504.
- Shin HC and Raymond SA (1991) Excitability changes in C fibers of rat sciatic nerve following impulse activity. *Neurosci Lett* 129:242-246.
- Shin HC (1996) Information decoding by axonal bifurcation. *Proceed Kor Inst Elect Eng* 11:16-20.
- Waikar SS, Thalhammer JG, Raymond SA, Huang JH, Chang DS and Strichartz GR (1996) Mechanoreceptive afferents exhibit functionally-specific activity dependent changes in conduction velocity. *Brain Res* 721:91-100.
- Watanabe T and Simada J. (1971) Auditory temporal masking: an electrophysiological study of single neurons in the cat's cochlear nucleus and inferior colliculus. *Jpn J Physiol* 21:537-549.
- Weisenberger JM (1994) Vibrotactile temporal masking: Effects of multiple maskers. *J Acoust Soc Am* 95:2213-2220.
- Wiegand L and Krumbholz K (1999) Temporal resolution and temporal masking properties of transient stimuli: Data and an auditory model. *J Acoust Soc Am* 105:2746-2756.