65. *Effective Stimulation Parameters for the LTP Formation in the Superior Colliculus Slices from the Guinea Pig*

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In 1973, Bliss and Lomo found the long-term potentiation (LTP) in the hippocampus of rabbits which was maintained for several hours after a short period of tetanic stimulation.¹⁾ While LTP can now be easily demonstrated, the distribution of LTP in the brain, the mechanism underlying the phenomenon, and the functional significance of the LTP remain unclear.²⁾ However, LTP formation is interpreted as substantial increase in synaptic efficacy and so attracts great interest because of the possibility that the phenomenon might underlie some aspects of memory storage.³⁾ It was suggested that LTP might be a general synaptic plasticity for modifiable synapses. If that is so, it is expected that LTP can be reliably recorded in many parts of the central and peripheral nervous systems, including the superior colliculus (SC).

Over the past 15 years, research findings on the formation of LTP in the mammalian brain have mainly come from the hippocampus and the neocortex area. LTP formation in the superior colliculus has not been reported in the literature except for goldfish tectum.⁴⁾

In this experiment, we reported the first demonstration of LTP formation in the SC slice from the guinea pig and determined the optimal stimulation parameters for the LTP formation.

Method. Guinea pigs weighing between 200 and 300 g (one month old) were decapitated, and the brains quickly removed. Tissue blocks of SC were rapidly dissected out and cut sagittally into slices of between 400 and 600 μm thickness with a razor blade. Details of the slice preparation have been reported elsewhere.⁵⁾ Before beginning the experiments, the slices were preincubated for a minimum of 20 min in the standard medium (concentration in mM: NaCl 125, KCl 3, KH_2PO_4 1.25, CaCl_2 2, MgSO_4 1.3, NaHCO_3 26, D-glucose 10) equilibrated with 95% O_2 and 5% CO_2 (pH 7.4). Thereafter each slice was transferred to the recording chamber under a stereomicroscope and submerged completely in the perfusion medium. Each layer of the SC can be clearly visualized and identifiable under the stereomicroscope. The chamber was perfused continuously with the standard medium at a rate of 8 ml/min. The temperature in the chamber was kept at 35.5°C throughout the experiment. Glass microelectrode filled with 2 M NaCl was placed in the superficial grey layer (SGL) of the SC and bipolar silver wire electrode for stimulation was placed in the optic layer (OL). Square pulses of 100 μsec in duration (Stimulator, Nihon Koden SEN7103) were applied for test stimulation which were discharged at the rate of 0.5 Hz. Electrical activity was recorded by oscilloscope (Nihon Koden VC10). The evoked field potential recorded here has been reported to be postsynaptic in nature.⁵⁾

Results. Fig. 1 shows a typical example of the LTP formation in the SGL.

Fig. 1. The appearance of LTP in SGL of SC slice and the time course of a typical example of the LTP formation. Right panel shows the PSPs elicited in the SGL of SC slice after the stimulation to OL. (1) indicates the PSP of maximum amplitude with one test stimulus. In (2) the stimulus intensity was adjusted to evoke PSP for the amplitude to be about 1/3 of the maximum amplitude. (3) and (4) show potentiated PSPs 5 and 15 min after the tetanic stimulation (50 Hz, 20 sec), respectively. Furthermore (5) and (6) show more potentiated PSPs 10 and 20 min after the second tetanic stimulation, respectively. Left panel indicates the time course of LTP formation of the slice shown in the right panel. In the figure the adjusted amplitude of PSP in (2) of right panel was taken as 100%. At 'tet ↓', the tetanic stimulation was applied to OL.

By controlling the intensity of the stimulus, maximum amplitude of PSP was first noted (1 of right-hand panel in Fig. 1) and then the intensity of the stimulation was adjusted for the PSP amplitude to be about 1/3 of the maximum amplitude (2 of right-hand panel) before the experiments were started. Here we employed the tetanic stimulation at the frequency 50 Hz and 20 sec in duration and this adjusted amplitude of PSP was regarded as 100%. The PSP was increased to about 190% of initial amplitude 15 min after tetanic stimulation (4 of right-hand panel). Furthermore, second tetanic stimulation was delivered 25 min after the first tetanus. PSP was increased further to about 270% of initial amplitude 10 min after the second stimulation (5 of right-hand panel). Thereafter PSP was maintained in about 250% of initial amplitude (6 of right-hand panel). The left panel shows a time course of the LTP formation recorded from this preparation using 50 Hz and 20 sec tetanic stimulation.

The formation of LTP can be modified by the stimulation parameters such as frequency, duration, and intensity.²⁾ In this experiment, we adjusted the intensity of the stimulation so as to evoke the PSP at about 1/3 of the maximum amplitude which empirically proved most potentiated for the LTP formation. In our SC slice preparation LTP was not observed at the frequency of less than 30 Hz or more than 200 Hz. Fig. 2 represents the time course of the LTP formation in the SC slices stimulated for the 1, 10, and 20 sec in duration at 100 Hz (A) and for the 1, 10, 20, and 30 sec at 50 Hz (B), respectively. It can be seen from Fig. 2 that the PSP amplitude was increased to 178% of the control

Fig. 2. The occurrence of LTP after the tetanic stimulations at the frequency of 100 Hz and 50 Hz in different duration. In (A), tetanic stimulation at the frequency of 100 Hz was applied for 1 (○—○), 10 (■—■), and 20 sec (●—●) in duration. In (B), tetanus at the frequency of 50 Hz was applied for 1 (○—○), 10 (■—■), 20 (●—●), and 30 sec (▲—▲). Each plot indicates the average value of five slices. Standard error means for each plot are not indicated in the figure, but they were within 15% of the mean value.

value 15 min after tetanic stimulation at 50 Hz and 20 sec in duration and it was stabilized at this level thereafter. LTP formation was not so distinctly induced by the tetanic stimulation of 1 and 10 sec in duration. The rate of LTP formation was thus maximum for the tetanic stimulation of 50 Hz and 20 sec in duration.

Discussion. In the present experiment, the postsynaptic potential recorded in the SGL after the OL stimulation must be postsynaptic in nature⁵⁾ and elicited by the activation of retino-tectal pathway because the same type of potential could be recorded after the stimulation of optic nerve (figure not shown). The finding that LTP can be reliably recorded in the SC is important and significant because this is the first demonstration of this phenomenon in the SC of the mammalian brain. LTP can be induced by a wide range of tetanus frequency, intensity and duration. As for the duration of the tetanus, it has been reported in hippocampal slices that LTP was easily induced by a tetanus applied for 1 sec at 100 Hz.²⁾ Yamamoto and Sawada indicated that the duration of the tetanus was not an important parameter if it was larger than 1 sec in hippocampal slices.⁶⁾ In hippocampal slices a total of 100–200 pulses are required for inducing LTP and furthermore at lower frequencies of stimulation (below

50 Hz) a heterosynaptic response depression can be seen following the termination of the tetanus,⁷⁾ and at higher frequencies of stimulation (between 100 and 400 Hz) such heterosynaptic depression is rarely observed.⁸⁾ On the contrary, our experiments of SC slice indicated that the tetanic frequency of 50 Hz and 20 sec in duration was most effective for the LTP formation and a total of 1000 pulses were required for the formation of distinct LTP.

It has been reported that the NMDA receptor, a subtype of glutamate receptor may be involved in the formation of LTP.^{2),9)} For testing this possibility in SC slice, we studied the effect of DL-2-amino-5-phosphonovalerate (APV), a specific antagonist against NMDA receptor, in order to know whether or not it might inhibit the appearance of LTP in SC. In the preparation of SC, a large dose of APV (500 μ M) inhibited the formation of LTP, showing that NMDA receptor may also be involved in the LTP phenomenon in SC. In the present study, the distinct LTP formation was observed in the SGL of SC. The details of mechanism of LTP formation in SC, however remain to be elucidated by further studies.

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