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Research Paper

Different effects of 2 and 100 Hz tetanus on the expression of long-lasting long-term potentiation in rat visual cortical slices

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Abstract: Long-term potentiation (LTP) can be induced by various tetanic parameters in the mammalian visual cortex. However, little researches have been done on the relationship between the expression of the long-lasting LTP (late phase LTP or L-LTP) lasting more than 3 h and the tetanic parameters. In the present study, the effects of 2 Hz and 100 Hz tetanic parameters on L-LTP of the field potentials were recorded from the layer Ⅱ/Ⅲ of the rat visual cortical slices in response to stimulation of the layer Ⅳ. As a result, tetanic parameters that had more than 300 pulses reliably induced L-LTP in the postnatal day 15~21 rats. Obviously different L-LTP expressions were induced by 2 Hz and 100 Hz tetani. There was no difference in L-LTP expression induced by the parameters with the same frequency and different total pulses. These data suggest that L-LTPs induced by different frequency parameters may have different induction and maintenance mechanisms; L-LTPs induced by the parameters with the same frequency may have the same mechanisms.

Key words: long-term potentiation; visual cortex

2 Hz 与 100 Hz 刺激在诱导大鼠视皮层长持续长时程增强中的不同作用

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摘 要: 在哺乳动物的视皮层, 多种不同参数的刺激可诱导出长时程增强(long-term potentiation, LTP)现象。但关于刺激参数与持续时间长于 3 h 的长持续 LTP (long lasting LTP, L-LTP)之间关系的研究较少。本研究用 3 周龄的大鼠视皮层脑片标本, 在Ⅳ层刺激而在Ⅱ/Ⅲ层记录场电位, 待场电位稳定后施加强直刺激诱导 LTP, 探讨2 Hz 与 100 Hz 的强直刺激在诱发持续时间长于 3 h 的 L-LTP 中的作用。结果表明, 多于 300 个脉冲不同频率的刺激可稳定地诱导出 L-LTP; 2 Hz 与 100 Hz 的刺激诱发的 L-LTP 有明显不同的表达形式, 100 Hz 刺激可诱导出较大的 L-LTP; 频率相同而脉冲数不同的强直刺激诱发的 L-LTP 有相同的表达形式。以上结果提示, 不同频率的强直刺激诱发的 L-LTP 机制可能不同; 相同频率的刺激(脉冲数不同) 诱发的 L-LTP 可能有相同的机制。

关键词: 长时程增强; 视皮层
中图分类号: Q42; R338.8

Activity-dependent changes in synaptic strength such as long-term potentiation (LTP) are believed to be critical for information processing and for the refinement of neuronal connections during development[1]. LTP consists of early and late stages according to the maintenance mechanisms: early phase LTP lasting less than 3 h and late phase LTP (L-LTP) lasting longer than 3 h[2-4]. It has been suggested that L-LTP is dependent on the transcription of mRNA and the synthesis of proteins[4,5]. In the developing visual cortex, LTP is analogous to the process of activity-dependent refinement of synaptic formation[6]. Experimentally, LTP in the visual cortex is often elicited by 2 and 100 Hz tetanus[7]. Interestingly, the 2 Hz tetanus that induces LTP in the visual cortex actually induce long-term depres-

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sion (LTD) in the hippocampus[7-9], suggesting that different brain areas may differ in their responses to stimulating parameters.

Numerous studies support the idea that visual cortex LTP underlies an experience-dependent modulation of visual functions such as the ocular dominance plasticity [6]. At the same time, it is suggested that patterned activity plays key role in modulating synaptic competition and pruning [10]. Patterns of visual activity are important for the development of visual cortex. However, there is no report regarding the relationship between tetanic parameters and properties of L-LTP in the visual cortex, such as induction, expression and maintenance. Here we used field potentials recorded in the layer Ⅱ/Ⅲ of visual cortical slices to examine the effectiveness of 2 and 100 Hz tetanus on the induction of L-LTP in the developing rat.

1 MATERIALS AND METHODS

1.1 *Slice preparation*. Sixty-four visual cortical slices were prepared from 64 male Sprague-Dawley rats at postnatal day 15~21(P15~21). Rats were housed in a standard environment on a 12:12-h light/dark cycle with lights on 07:00, and allowed access to water and food *ad libitum*. Dissections were consistently performed between 9:00 and 11:00 am. Rats were initially anesthetized with ether and then immersed in ice-cold water except for the nose for 3 min to reduce brain temperature. Immediately after decapitation, the brain was removed in an ice-cold artificial cerebral spinal fluid (ACSF) bubbled with 95% O_2 and 5% CO_2. The ACSF contained (in mmol/L) NaCl 124, KCl 5, KH_2PO_4 1.25, $MgSO_4$ 2, $CaCl_2$ 2, $NaHCO_3$ 26, and glucose 10 (pH 7.40). A block of tissue containing the visual cortex was cut into 400 μm thick slices with a vibratome (Campden Instruments, London, UK). The slices were transferred to an interface type recording chamber maintained at 31ºC and incubated for at least 1.5 h prior to electrophysiological recording. This study was carried out in compliance with the Guide for the Care and Use of Laboratory Animals at the Medical School of Xi'an Jiaotong University. All procedures were conducted with the minimum number of animals necessary for data collection and with minimal stress or pain in the subjects.

1.2 *Electrophysiology*. To record in the monocular (Oc1M) and binocular (Oc1B) subfields of the rat primary visual cortex, only slices matching sections pictured in Zilles were used[11]. The extracellular field excitatory postsynaptic potentials (fEPSPs) were recorded using micropipettes filled with 2 mol/L NaCl solution with impedances of 1.0~2.0 MΩ. A monopolar recording configuration was employed with the slice pool grounded. Electrical stimulation was administered via a 75 μm bipolar concentric stimulating electrode. With the aid of a binocular microscope, the recording electrode was placed in layer Ⅱ/Ⅲ. The stimulating electrode was placed in layer Ⅳ. Data were collected and analyzed using an A/D converter (Powerlab 200; ADInstrument, Australia) and a personal computer. Once the electrode was placed, a recording session began with a determination of the threshold stimulation level which obtained a 200 μV synaptic response[12]. Slices that exhibited thresholds greater than 50 μA were considered 'unhealthy' and were not used for recording. The stimulus intensity was set to evoke a response of ~50% of the maximum amplitude. Test stimuli of 0.1-ms duration were delivered once per minute. The test stimulus was applied throughout the experiments, except during the tetanus. Baseline recordings were collected for at least 20 min prior to experimental manipulations, to assure stability of the responses[13]. After the stable baseline recording was obtained, the tetanus protocol was applied at the test intensity. 2 Hz and 100 Hz tetani were used. Details of tetani were listed in Table 1. Slices in which post-tetanus fEPSPs slope received and maintained at a level at least 20% higher than average fEPSPs slope in pre-tetanus baseline recording for up to 3 h were demonstrated as L-LTP. Those that decay to baseline in 3 h were said to be LTP (<3 h).

1.3 *Statistical evaluation*. The data are presented as means±SEM. The incidences of L-LTP induced by two tetanic parameters were compared by chi-square test. For multiple comparisons, one-way analysis of variance was used. Comparison between two experiment groups was made by the unpaired Student's *t* test. *P* values <0.05 were considered to have statistical significance.

2 RESULTS

A typical fEPSPs elicited in layer Ⅱ/Ⅲ in response to layer Ⅳ stimulation consists of two major components: a negative wave with peak latency at 3.5~4.0 ms and a second negative component with peak latency at 5.5~7.5 ms (Fig. 1*A*). Those components were thought to present synaptic activity because they were eliminated by Ca^{2+} free medium or by the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX ,10 μmol/L) (Fig.1*A*). The first negative component was considered to be monosynaptic response because of its short latency[12].

We used the first negative-going potential as index for assessing synaptic modification induced by tetanus. The expression of LTP was described in Fig. 1*B*. After tetanus, fEPSPs progressively increased until 60 min post-tetanus when maximum potentiation was achieved and remained without decrement throughout the observation period.

To evaluate the relationship between the expression of LTP and tetanic parameters, various tetani were used. From the results, we found that parameters with more than 300 pulses could steadily induce L-LTP (Table 1). L-LTP induced by 100 Hz tetanus had higher mean fEPSP slope than that induced by 2 Hz tetanus (Table 1 and Fig. 1*B*). At the same time, we compared the different effects of the parameters with the same frequencies and different total pulses. We found that 2 Hz tetanic parameters with 1 200 total pulses had higher incidence of L-LTP than 300 pulses and there was no difference in the mean fEPSP slopes between those two groups (Table 1 and Fig. 1*B*).

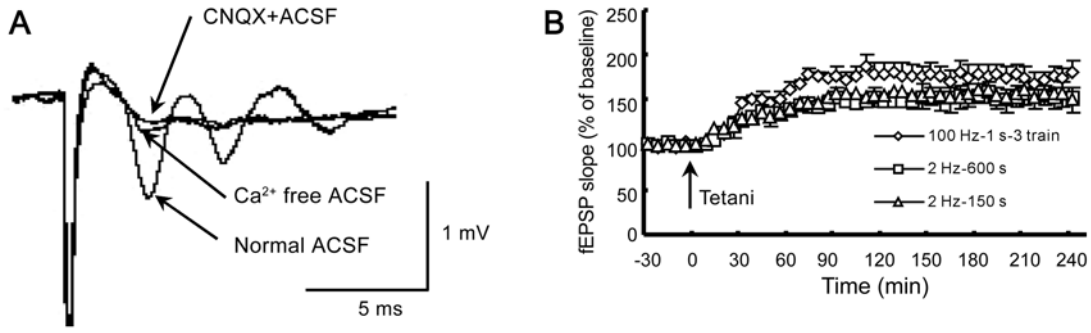

Fig. 1. Expression of LTP in visual cortex. *A*: Evoked potentials in normal, Ca^{2+}-free, and CNQX-containing ACSF. Initial downward deflection is stimulation artifact. *B*: Time course of long-lasting LTP induced by various tetani.

Table 1 L-LTP induced by various tetanic parameters at P15~21

Frequency (Hz)	Duration (s)	Trains	Total number of pulses	Total cases (n)	Induction of L-LTP Incidence	Induction of L-LTP Mean slope (%)	Induction of LTP Incidence	Induction of LTP Mean slope (%)
2	600	1	1200	16	87.5% ▲	144.83 ± 2.15%	0	–
2	150	1	300	10	40%	146.09 ± 2.20%	0	–
2	50	1	100	10	20%	142.25 ± 13.74%	30%	135 ± 2.1%
100	1	3*	300	16	81.25%	166.12 ± 3.34%△	0	–
100	1	1	100	12	–	–	60%	155 ± 3.54%

*Three trains of 100 Hz delivered with 30-s train intervals. △P<0.01 compared with 2 Hz groups. ▲P<0.01 compared with 2 Hz-150 s and -50 s groups.

3 DISCUSSION

In the vast researches on LTP, numerous tetanic parameters were used. They had different frequencies, intensities and stimulating patterns. In the hippocampus, high frequency stimulations such as 100 and 200 Hz stimulating parameters were frequently used to elicit LTP. Another tetanus such as theta burst stimulation (TBS) was thought to be particularly important in the hippocampus because it is a more modest stimulus parameter and a synchronized firing pattern at similar frequencies occur in the hippocampus during learning[14].

As a cellular model for activity-dependent synaptic plasticity, synaptic modification and refinement of cellular connection, LTP in visual cortex represents some developing processes of neuron[15]. Impulse activity is important for the topographic refinement of synaptic connections within the developing visual system, and the spontaneous impulse activity has been shown to be important in the formation of ocular dominance columns in visual cortex[16,17]. It is also suggested that patterned activity plays key role in modulating synaptic competition and pruning[10]. Most of the tetanic parameters used to induce LTP in visual cortex were those used in hippocampus. TBS is also used and is thought to be an effective stimulation protocol for neocortical potentiation[18,19]. However, no matter in the hippocampus or in the visual cortex, it is not clear whether patterns of activity that induce LTP and LTD occur during learning or mimic *in vivo* neuronal activity. There are supports for the view that low frequency stimu-

lation may mimic physiological activity during development, and thus a protocol of low frequency stimulation may contribute to the structural refinement of cortical circuit[16,20]. From this point, 2 Hz tetanus is more significant than 100 Hz tetanus. From our results, 100 Hz tetanus induced a significantly larger L-LTP in the mean slope of the fEPSPs (% of baseline) than 2 Hz tetanus did. Recent studies have indicated that the intracellular postsynaptic Ca^{2+} rise and the subsequent synaptic plasticity are influenced by the different frequency tetani[21,22], which lead to activation of different calcium channels[23,24]. Stimulations with different frequencies can induce different expressions of mRNA and different transmitter release[23,25]. Taken together, these data suggest that there are different induction and maintenance mechanisms in the L-LTP induced by different frequency parameters.

Our results also show that there is no difference between 2 Hz tetanus groups with different pulses in the expression of L-LTP and that tetanus with more pulses has higher priority in inducing L-LTP. This suggests that there are same induction and maintenance mechanisms underlying the L-LTP induced by 2 Hz tetanus, no matter how many the total pulses are.

From the results, we can conclude that the frequency of tetanus plays a key role in the induction of L-LTP in rat primary visual cortex. L-LTP induced by different frequency parameters maybe share different induction and maintenance mechanisms; when induced by the same frequency, they may be the same.

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