Changes of synaptic structure after long-lasting LTP induced by high and low frequency tetanus in slices of the rat visual cortex

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Abstract: Synaptic ultrastructural changes after long-lasting long-term potentiation (L-LTP) induced by 2 and 100 Hz tetanus were investigated by electron microscopic and stereological approach in slices of the developing rat visual cortex (postnatal days 15~21). Both 2 and 100 Hz tetanus-induced L-LTP groups showed significant increases in synaptic interface curvature, synaptic numeric density and postsynaptic density thickness, as well as significant decreases in the cleft width, as compared with the control groups. In addition, the volume density of the active zone (AZ) was increased significantly in the 100 Hz tetanus-induced L-LTP group, but not in the 2 Hz group. The mean lateral area of individual AZ in the 100 Hz group was relatively higher than that in the 2 Hz group. These data suggest that newly formed synapses in the 100 Hz tetanus-induced L-LTP group are larger than those in the 2 Hz group and that 100 Hz tetanus might trigger reorganization or synthesis of postsynaptic cytoskeleton.

Key words: long-term potentiation; visual cortex; morphology; synapse
been suggested that L-LTP is dependent on the transcription of mRNA and the synthesis of new proteins. Visual cortex LTP was observed in slices from rats during a postnatal critical period but disappeared thereafter. Those observations support the idea that visual cortex LTP underlies an experiment-dependent modulation of visual functions such as the ocular dominance plasticity. Experimentally, LTP in the visual cortex is often elicited by 2 and 100 Hz tetanus. Interestingly, the 2 Hz stimulation that induces LTP in the visual cortex actually induces long-term depression in the hippocampus, suggesting that different brain areas may differ in their responses to the same stimulating parameters. In our previous study, we found that there is clearly different L-LTP expression between 2 and 100 Hz induced L-LTP.

Recently, studies have shown that synapses are extremely dynamic structures, changing both functionally and morphologically in response to activities. Indeed, induction of LTP initiates a sequence of morphological changes in the hippocampus, and application of high-frequency tetanus that induces LTP may trigger the growth of filopodia or even dendritic spine-like structures. However, no previous work has been done to examine whether LTP-associated morphological changes differ in response to LTP induction by different tetanus. To address this question, we herein observed changes of synaptic ultrastructure following L-LTP induced by 2 and 100 Hz tetanus in slices of the developing rat visual cortex.

1 MATERIALS AND METHODS

1.1 Slice preparations

Visual cortical slices were prepared from male Sprague-Dawley rats at postnatal days 15–21. Rats were housed in a standard environment on a 12h/12h light/dark cycle with lights on 07:00 am, 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, immersed in ice-cold water (nose exposed) for 3 min to reduce brain temperatures, and decapitated. Brains were removed immediately and placed into ice-cold artificial cerebral spinal fluid [ACSF (mmol/L): NaCl 124, KCl 5, KH₂PO₄ 1.25, MgSO₄ 2, CaCl₂ 2, NaHCO₃ 26, and glucose 10 (pH 7.40)] bubbled with 95% O₂ and 5% CO₂. Blocks of tissue containing the visual cortex were cut into 400 µm thick slices with a vibratome (Campden Instruments, London, UK), transferred to an interface recording chamber maintained at 31°C and incubated for at least 1.5 h prior to initiation of electrophysiological recordings. 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

Slices matching sections pictured in Zilles were used for recordings in Oc1M or Oc1B. The extracellular field excitatory postsynaptic potentials (fEPSPs) were recorded using micropipettes filled with 2 mol/L NaCl (impedances of 1.0–3.0 MΩ). A monopolar recording configuration was employed and the slice pool was grounded. Electrical stimulation was administered via a concentric stimulating electrode. With the aid of a binocular microscope, the recording electrode was placed in layer I/II and the stimulating electrode was placed in layer IV of area 17. Data were collected and analyzed using an A/D converter (Powerlab 200; ADInstruments, Australia) and a personal computer. The stimulus intensity was set to evoke a response of 50% of the maximum amplitude. All stimulatory pulses were 0.1 ms in duration. One stimulatory pulse was delivered every 1 min for at least 30 min to provide a pre-tetanus fEPSPs baseline for comparison to the post-tetanus responses. After the baseline data were collected, experimental slices received tetanic stimulation and fEPSPs were recorded once every 1 min for at least 4 h. We defined LTP as an increase larger than 20% in the average fEPSPs slope of the responses, relative to the baseline. All slices were divided into four groups: slices incubated in recording chamber for 4 h (group 1), slices that were only given test stimulus (group 2), slices with L-LTP induced by a tetanus at 2 Hz for 10 min (2 Hz-10 min; group 3), and slices with L-LTP induced by three trains of 100 Hz for 1 s at an interval of 30 s (100 Hz -1 s -3 trains; group 4).

1.3 Electron microscopy

Visual cortex samples were fixed about 4 h after L-LTP induction and then processed for electron microscopy. Briefly, slices were fixed overnight at 4°C in 3% glutaraldehyde, and then rinsed in 0.1 mol/L phosphate buffer (pH 7.4). The blocks of slices that were properly below the recording electrode were post-fixed for 2 h in a fresh solution of 1% osmium tetroxide, serially dehy-
drated in ethanol and embedded. Sections were stained for 45 min in 0.5% uranyl acetate and 45 s in lead citrate before being observed under a Hitachi H-600 electron microscope (10 000–40 000×).

1.4 Morphological analysis

The morphological analysis was processed according to our previous experiment[20]. Gray type I synapses were identified by the presence of pre- and postsynaptic membrane apposition, synaptic clefts, postsynaptic densities (PSD), and clusters of at least three vesicles near the presynaptic membrane[20]. Synapses were further divided into four types according to the observed synaptic curvature: convex synapses, in which the postsynaptic element protrudes into the presynaptic terminal; concave synapses, in which the presynaptic element protrudes into the post synaptic element; flat synapses; and irregular synapses, characterized by more than one curvature and including perforated synapses (Fig.1).

Fig. 1. Electron micrographs show the different types of synapses. A: Convex type of synapse with presynaptic vesicles (open arrow), presynaptic active zone (triangle) and postsynaptic density (closed arrow). B: Concave type of synapse with synaptic cleft (arrow). C: Flat type of synapse. D: Perforated synapse. Scale bar, 200 nm.

For morphometric analysis, the following structural parameters were examined using a graph analyzer (Leica, Germany): the width of synaptic cleft (the mean of three measurements), the thickness of the PSD at its thickest part, the length of the active zone (AZ), and the curvature of the synaptic interface. The synaptic numeric density (Nv) was calculated using the following stereological equation: Nv=ΣQ−/Σa·h, where Q− is the number of unique counting objects in one photographic section, a is the area of the counting frame, and h is the height of the physical disector [21]. Another synaptic parameter analyzed was the AZ membrane area per unit tissue volume, also known as the volume density of the AZ (Sv), which was calculated according to the formula Sv=(4/π) ×Lα where Lα is the mean total length of active zone profiles per unit area of section. Combining estimates of Sv and Nv allowed the unbiased estimation of the mean lateral area of individual AZ (Sαz) by the formula Sαz= Sv / Nv[22].

1.5 Statistical analysis

The structural parameters are presented as mean± SD and the mean increase of fEPSPs slope is presented as mean±SEM. Chi-square tests were applied for statistical comparison of the number of synaptic types and the incidences of L-LTP induced by the two tetanus parameters. For multiple comparisons, one-way analysis of variance was used. Student-Newman-Keuls test or unpaired t-test was used to determine the significance of the differences between the two groups. Probability values of P<0.05 were considered as the indication of statistical significance.

2 RESULTS

Following application of tetanus, the recorded fEPSPs slope progressively increased until about 60 min post-tetanus. At this time, maximum potentiation was achieved; fEPSPs slope remained maximal from that point on, without decrement, throughout the observation period. No difference was observed in the induction incidences (14 out of 16 versus 13 out of 16, P>0.05, χ²), but there was a significant difference in the mean increase of fEPSPs slope [(144.83±2.15)% versus (166.12±3.34)%, P<0.05, t-test] of LTP induced by 2 and 100 Hz tetanus (Fig. 2).

There were no differences between the two control groups for all the tested synaptic parameters (Table 1 and Fig.3), and there was no significant difference in any group as regards the percentage of synaptic types represented in the samples (Fig. 3). However, in terms of the tested parameters, the L-LTP (2 and 100 Hz) groups differed significantly from control groups in the width of the synaptic cleft, the thickness of PSD, the curvature of the synaptic interface and Nv. Both 2 and 100 Hz tetanus-induced L-LTP groups showed significant increases in synaptic
interface curvature, Nv and postsynaptic density (PSD) thickness, as well as significant decreases in the cleft width, as compared with the control groups. Interestingly, the Sv and mean lateral areas of individual AZ in the 100 Hz induced group were significantly higher than those in 2 Hz group.

3 DISCUSSION

Patterned activity is thought to play key roles in modulating synaptic competition and pruning. Previous work has suggested that low-frequency stimulation mimics physiological activity during development, and thus a protocol of low-frequency stimulation may contribute to the structural refinement of cortical circuits. Recent studies have indicated that the intracellular postsynaptic Ca<sup>2+</sup> rise and the subsequent synaptic plasticity is influenced by the different tetanus, and different frequency stimulations lead to activation of different calcium channels and different transmitters release. A number of studies have approved that synaptic structural modifications are associated with LTP expression. Therefore, L-LTP induced by 2 and 100 Hz protocols may trigger different synaptic ultrastructural changes.

The present results show that there are several common features of L-LTP induced by 2 and 100 Hz tetanus. Consistent with the results of previous studies, we found that curvature and total synaptic number per unit volume were increased with the maintenance of L-LTP induced by 2 and 100 Hz tetanus. The proportions of different synaptic types did not change significantly after L-LTP formation in any group, whereas the mean lateral area of the AZ decreased following L-LTP induction. This result is consistent with previous data, which have shown that synapses newly formed after LTP are smaller than previously established synapses.

In addition to the consistent changes of synaptic parameters between 2 and 100 Hz tetanus-induced L-LTP, we observed differences in the changes in the Sv and the mean lateral area of individual AZ between the two groups. Sv and the mean lateral area of individual AZ in the 100 Hz group were relatively larger than those in the 2 Hz group, suggesting that newly formed synapses in the 100 Hz group had larger individual AZ than those in the 2 Hz group. Our
previous study shows that L-LTPs induced by 2 and 100-Hz tetanus may be produced by presynaptic and postsynaptic mechanisms respectively\[31\]. At the same time, various data show that postsynaptic cytoskeleton such as tubulin and actin play important roles in the synaptic morphological changes following LTP induction\[32\]. There is a long-lasting increase in actin content within dendritic spines after L-LTP induction and inhibition of actin polymerization impairs L-LTP without affecting the initial amplitude and early maintenance of LTP\[33\]. From these data, we suppose that 100 Hz tetanus might trigger reorganization or synthesis of postsynaptic cytoskeleton and then makes the newly formed synapses larger than those in the 2 Hz group. More studies are needed to demonstrate this hypothesis.

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