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Different patterns of changes between actin dynamics and synaptic density in the rat's primary visual cortex during a special period of visual development

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ABSTRACT

In our previous study, we found that the normalized levels of the synaptosomal filament actin (F-actin) to monomeric global actin (G-actin) ratio in the primary visual cortex (V1) of rats was significantly lower on postnatal day (P) 45 compared with P30, however, the synaptic density in the monocular area of primary visual cortex (V1M) maintained a stable high level from P30 to P45. The mechanisms underlying the different patterned of change in synaptic density and actin rearrangements from P30 to P45 are unclear. During visual development, there is a synaptic pruning process in the binocular segment of primates' visual cortex (V1B) and we suppose the pruning activity may contribute to the decreased synaptosomal F-actin to G-actin ratio. To address this issue, first, samples were derived from the region of V1B for TEM analysis but no significant difference was demonstrated between the P30 and P45 groups. In addition, the expression of PSD-95 detected by immunobloting in the synaptosomes of V1 at P30 and P45 also showed no significant difference. Combined with the previous results of actin dynamics in the V1 and synaptic density in the V1M, we conclude that the synaptic density and actin dynamics in the rats' primary visual cortex are inter-related but not absolutely identical. This study suggests actin cytoskeleton not only provides the structural basis but also regulates a various array of cellular activities underlying synaptic function. Besides, it highlights a further research of synaptic pruning.

1. Introduction

In an unpublished study of our previous experimental results (in submission), using transmission electron microscopy (TEM), we found an increased synaptic density in the cortical layer IV–VI of the monocular area of rat's primary visual cortex (V1M) at postnatal day (P) 30 compared with P15 and maintained a stable high level from P30 to P45. Actin is the primary component of synaptic filaments. During visual development, the normalized levels of the synaptosomal filament actin (F-actin) to monomeric global actin (G-actin) ratio in the primary visual cortex (V1) also peaked at approximately P30, however, the ratio was significantly lower at P45 compared with P30 (Fig. 1A). The mechanisms underlying this different patterned of change in synaptic density and actin rearrangements from P30 to P45 are still unclear.

Two potential mechanisms may play roles in the occurrence of the phenomenon: First, a synaptic pruning process happens in the primates' binocular segment of the visual cortex (V1B) during visual development (Hubel et al., 1977; Luo and O'Leary, 2005), which is essential for the maintenance of accurate synapses derived from the eye-specific patterned of connections (Chiu and Cline, 2010; Colman et al., 1997; Hashimoto and Kano, 2003). For rat and mouse, the medial two-third of the V1 region receives information from the single contralateral eye and belongs to V1M, whereas the lateral one-third of the V1 region receives information from both contralateral and ipsilateral eye and is called of V1B (Fig. 1B) (Gordon and Stryker, 1996). Previously the samples we investigated the normalized levels of the F-actin to G-actin ratio were

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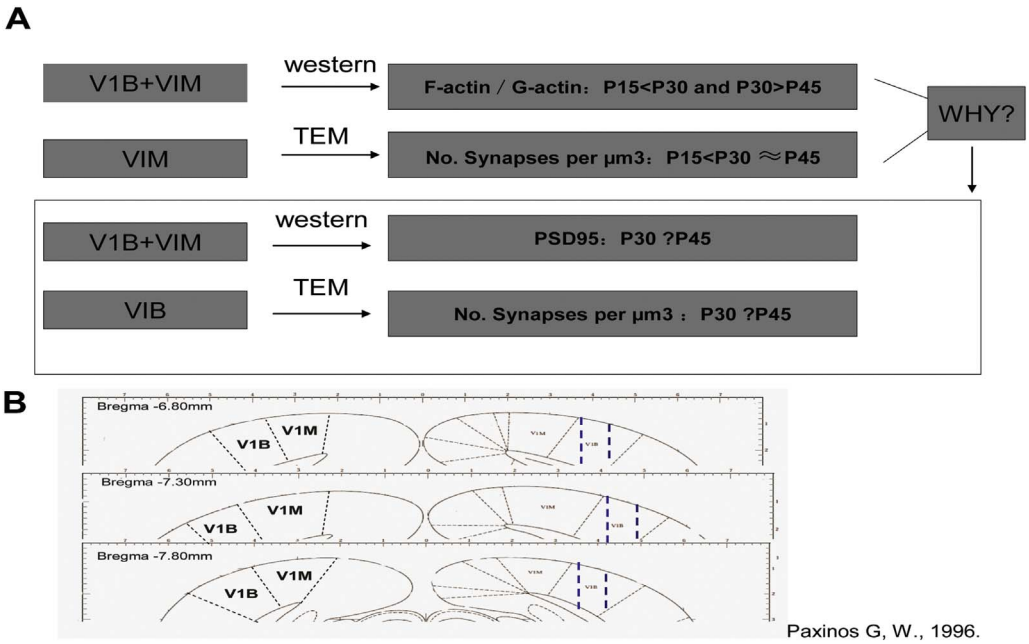

Fig. 1. (A) Shows the background of the present research and a schematic representation of the experimental design. (B) The composition of the primary visual cortex (V1) in rat is shown.

derived from the V1 (contains both the V1M and V1B), whereas the samples in synaptic density analysis were from the cortical layer IV–VI of the V1M. Whether the decrease of F-actin to G-actin ratio from P30 to P45 reflect the synaptic pruning process in V1B need further investigation. Second, besides constituting the synapse structure, filamentous actin may be present near synaptosomal membranes and its dynamic changes may reflect the changes in the functional visual synaptic plasticity (Cingolani and Goda, 2008). Therefore, the results of synaptic density and actin rearrangement may be not absolutely identical, which also need additional exploration.

To address whether the synaptic density in the cortical layer IV–VI of V1B decrease from P30 to P45, first, the samples were derived from the region of V1B for TEM analysis. On the other hand, to address whether the synapse decrease occur in the synaptosomals of those for detecting the F-actin to G-actin ratio, we detect the expression of the PSD-95 in the synaptosomals of the V1 region.

2. Materials and methods

2.1. Subjects

S-D rats (between postnatal days 15 and 45) were kept at 20 °C–24 °C on a 12 h light/dark cycle with food and water available ad libitum. All experiments conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Ethics Committee of Eye Institute of Shandong University of Traditional Chinese Medicine.

2.2. Drugs and antibodies

Sources of antibodies are as follows. Rabbit anti-actin, Rabbit anti PSD-95, and Rabbit anti GAPDH antibody were obtained from Sigma-Aldrich (St. Louis, USA). Goat anti-rabbit secondary antibody was purchased from Jackson Immuno Research (Pennsylvania, USA).

2.3. Tissue preparation for TEM

In the present research, a three-step localization method was employed to accurately confine TEM samples to the binocular region of the primary visual cortex. The method was described in previous reports (Bi et al., 2010; Bi et al., 2015). Briefly, rats were perfused with 0.9% NaCl after deeply anesthetized, followed by phosphate-buffered fixative containing 2% paraformaldehyde and 2.5% glutaraldehyde. The first step is anatomical localization: The V1B was identified based on anatomical landmarks according to the atlas (Paxinos, 1996). Each 3-mm-long V1B slice, with a section area of 1 mm^2 containing cortical layers I to VI, was dissected out, post-fixed, treated with osmium tetroxide, dehydrated, and embedded (Fig. 1B). Each sample for electron microscopy was taken in a same location. The second step is histological localization: 2-μm-thick semi-thin sections were cut from the long side of each embedded slice, also containing cortical layers I to VI. The third step is ultrastructural localization: Serial 70-nm-thick ultrathin sections adjacent to the semi-thin sections were sliced and used for ultrastructural localization. Seven to nine pieces of serial ultrathin sections were collected on a single-slot grid.

2.4. Data analysis for electron micrographs

Choosing areas that contained neuropil around the large pyramidal neurons of layer IV–VI on serial ultrathin sections of each single-slot grid were used for photographing and statistics. Ten non-contiguous visual fields with which a visual field covering neuropil regions of 250 μm^2 from each ultrathin slice were imaged at 10,000 × magnification and used for quantification. Synapses were defined as the ultrastructures containing a presynaptic membrane, a postsynaptic density, and presynaptic vesicles. The density of synapses per μm^3 was estimated as described previously using the formula $N_V = N_A/d$ (Alonso-Nanclares et al., 2008; Beaulieu and Colonnier, 1985; Bi et al., 2015). N_v is the number of synaptic profiles per unit volume, N_A is the number of synaptic profiles per unit area and d is the average length of synaptic junctions, respectively. Image J software was employed to determine both N_A and d. The average of the visual fields obtained for each sample was calculated and is expressed as the mean per animal. Each group contained at least 3 animals.

2.5. Synaptosome preparation

After fresh rat brains were rapidly removed, coronal brain sections (1-mm-thick) were collected using an equipment of brain matrix (Reward Life Science, Shenzhen, Guangdong, China). According to the atlas (Paxinos, 1996), blocks of the V1 were obtained from the brain slices at 0 °C using a razor blade and were then frozen in liquid