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Relationship between monocularly deprivation and amblyopia rats and visual system development

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ABSTRACT

Objective: To explore the changes of lateral geniculate body and visual cortex in monocular strabismus and form deprived amblyopic rat, and visual development plastic stage and visual plasticity in adult rats. **Methods:** A total of 60 SD rats ages 13 d were randomly divided into A, B, C three groups with 20 in each group, group A was set as the normal control group without any processing, group B was strabismus amblyopic group, using the unilateral extraocular rectus resection to establish the strabismus amblyopia model, group C was monocular form deprivation amblyopia group using unilateral eyelid edge resection + lid suture. At visual developmental early phase (P25), meta phase (P35), late phase (P45) and adult phase (P120), the lateral geniculate body and visual cortex area 17 of five rats in each group were exacted for C-fos Immunocytochemistry. Neuron morphological changes in lateral geniculate body and visual cortex was observed, the positive neurons differences of C-fos expression induced by light stimulation was measured in each group, and the condition of radiation development of P120 amblyopic adult rats was observed. **Results:** In groups B and C, C-fos positive cells were significantly lower than the control group at P25 ($P<0.05$), there was no statistical difference of C-fos protein positive cells between group B and group A ($P>0.05$), C-fos protein positive cells level of group B was significantly lower than that of group A ($P<0.05$). The binoculus C-fos protein positive cells level of groups B and C were significantly higher than that of control group at P35, P45 and P120 with statistically significant differences ($P<0.05$). **Conclusions:** The increasing of C-fos expression in geniculate body and visual cortex neurons of adult amblyopia suggests the visual cortex neurons exist a certain degree of visual plasticity.

1. Introduction

Amblyopia is a visual impairment in poor space vision syndrome because of various unfavorable factors in the sensitive period of visual development[1]. Studies have reported[2], younger patients with amblyopia often have better treatment effect, curative effect are poor in adults. Other studies showed that[3], mammalian neural connections and synaptic structure can be adjusted according to the environmental stimuli after birth, this crucial period is known as the visual developmental plasticity phase, which ends in animals become adult[4-8]. To explore the changes of lateral geniculate body and visual cortex in amblyopic rat aged 13 d, established monocular strabismus and form deprived models, then observed the changes of lateral geniculate body and visual cortex at different visual development time points and discussed the visual plasticity in different development and adult. The results are reported as follows.

2. Materials and methods

2.1. Experimental animal

A total of 60 healthy SD rats of clean grade ages 13 d were selected, male and female unlimited, weight 16.5-26.3 g, average (21.4±2.93) g , provided by the animal experiment center. All the rats were kept at room temperature (23±3) ℃, humidity 50%-70%, with free access to food and water, animal illumination of 30 Lx, intensity of illumination for breeding work light of 200 Lx, alternating light and shade from 12 h/12 h to 14 h/10 h, Experiments on animals process were strictly follow the administration regulations of experimental animals.

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2.2. Instruments and reagents

GT-2000NV Visual evoked potentiometer and SOM20OD Surgical Microscope were provided from Chongqing China Medical Equipment Co., LTD; SABC IHC kits and DAB clour reagent were provided from GE Healthcare; OlymPus biological microscope and Leica Image system gel image analysis software were provided from Leica; mouse anti rat C-fos McAb was provided from ABCAM, UK; compound pyrazole, gentamycin, ketamine hydrochloride injection and chlorpromazine hydrochloride injection were provided by the Beijing Yongkang Pharmaceutical Co., LTD.; chloral hydrate and paraformaldehyde were provided by Guangzhou Chemical Reagent Factory.

2.3. Model establishing and grouping

A total of 60 SD rats were randomly divided into A, B, C three groups with 20 in each group, group A was set as the normal control group without any processing, group B was strabismus amblyopic group, using the unilateral extraocular rectus resection to establish the strabismus amblyopia model. Model was established in respective left and right eyes of 10 each 10 rats, after chloral hydrate anesthesia, eyelid routine disinfection was carried out along the bulbar conjunctiva and corneal limbus incision for separation extraocular rectus. The surrounding connective tissue and the lateral ligament were complete removed after resection to form esotropia, then the rats were fed with female rats together. After the wound healed, corneal reflection optical method was used to measure the strabismus degree, average diagonal 15 ℃-25 ℃, the model of group B was established by then. Group C was monocular form deprivation amblyopia group using unilateral eyelid edge resection+lid suture. After chloral hydrate anesthesia, and disinfecting eyelids, unilateral upper and lower eyelid were cut off 1 mm from internal to external rim. Mattress-suture was performed on the wound surface of the upper and lower eyelids line to close the eyelids of left and right eye respectively on 10 rats, then the rats were fed with female rats together. Amblyopia formation were determined in groups B and C of rats by flash visual evoked potential test, the model was established by then.

2.4. Experimental method

The lid was opened after electrophysiological testing in groups C; groups A and B were fed in the black box for 24 h. Five rats respectively were put under natural light at P25, P35, P45, P120 for 0.5 h to induce the C-fos protein expression in visual cortex. Chloral hydrate anesthesia was used in abdominal cavity, abdomen along the edge of the rib was opend to expose the heart and aorta; the infusion needle was injected by left ventricular upward into the artery, and needle by heart external end-actuator was fixed. After infusion, saline 70 mL was rapidly infused after right ear cut, at the end of infusion, 200 mL of 4% paraformaldehyde was added within 1 h perfusion. After infusion, beheaded method was used to remove the head quickly, all the brain tissue was extracted optic chiasma to the removal of the cerebellum, and repaired tissue block was fixed in formalin for 6 h. Tissue block was fixed in 10%, 20%, 30% sucrose successively. After sinking, the tissue block was cut into 10 μm thickness frozen section and sticked on the slide pre-processed by poly lysine. Ten sections were made from each specimen for immunohistochemical staining, C-fos protein expression of lateral geniculate body and visual cortex area 17 in each rat were observed.

2.5. Interpretation of results

Of each rat, 10 visions were randomly selected for immunohistochemical examination in lateral geniculate body analysis, C-fos protein positive cells in the average optical density and the percentage of positive particles were measured using the LeikaQ-Win image analysis system.

2.6. Statistical treatment

Using SPSS19.0 statistical software to deal with the data in terms of (mean± sd), t test was adopted, $P<0.05$ was considered statistically significant difference.

3. Results

3.1. Comparement of C-fos protein positive cells in geniculate body tissue of rats

C-fos protein positive cells of group A at P25 was the highest among groups, and gradually declined over time, in groups B and C, C-fos positive cells were significantly lower than the control group at P25 ($P<0.05$), there was no statistical difference of non-model C-fos protein positive cells between group B and group A ($P>0.05$), non-model C-fos protein positive cells level of group B was significantly lower than that of group A ($P<0.05$). The binoculus C-fos protein positive cells level of groups B and C were significantly higher than that of control group at P35, P45 and P120 with statistically significant differences (Table 1).

3.2. Immunohistochemical results of geniculate body

According to the microscope results of grouops B and C, the lateral geniculate body cells dominated by model eye were much smaller than that of contralateral eye, and Al area staining was light, lateral geniculate body cells of group A were normal (Figure 1).

3.3. PLI value comparison of C-fos positive cells in visual cortex area 17

PLI value comparison of C-fos positive cells of three groups were highest at P25, and gradually declined over