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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 binocular 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) °C, 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 SOM200D Surgical Microscope were provided from Chongqing China Medical Equipment Co., LTD; SABC IHC kits and DAB colour 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 °C–25 °C, 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 opened 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 stucked 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. Comparison 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 groups B and C, the lateral geniculate body cells dominated by model eye were much smaller than that of contralateral eye, and A1 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

time, PLI value of C-fos positive cells in group A at P35 was significantly higher than that of groups B and C; in groups B and C, PLI value comparison of C-fos positive cells in the same side eye cortex were significantly higher than that of group A, difference between groups was statistically significant ($P < 0.05$) (Table 2).

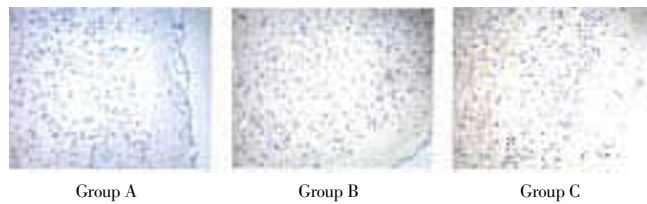


Figure 1. Immunohistochemical results of geniculate body ($\times 200$).

3.4. Immunohistochemical results comparison of visual cortex area 17

The electron microscope results showed that visual cortex neurons of group A were of the same size, cytoplasm swelled obviously, C-fos protein expression was hardly seen in adult rats of group A. C-fos protein in corresponding side of cortical neurons of groups B and C were significantly reduced than that of group A, the size of neurons was smaller, and the nucleus color deepened. There was no C-fos protein expression in groups B and C, a large number of C-fos protein were seen in the rest of the five layers with tan color as positive immune response (Figure 2).



Figure 2. Immunohistochemical results comparison of visual cortex area 17 ($\times 200$).

4. Discussion

Studies have shown that^[9], mammalian visual system are not mature at birth, gradually mature with external environment stimulation. In the early development of the visual system, the visual plasticity can be impaired into amblyopia due to factors such as visual deprivation, competition or inhibition^[10–15]. Visual evoked potential (F-VEP) is the electrical signals induced by visual pathways electrical activity in occipital visual cortex, after receiving corresponding stimulation in retina, it can objectively reflect the functional status of visual pathways, and play an important curative role in a wide range of applications, including strabismus and amblyopia diagnosis and judgment of curative effect^[16–18]. In this study, there was no significant difference of F-VEP during latency period and peak phase between a set of visual development periods and adult period, which is consistent with the visual mature time reported in literatures^[19].

C-fos gene is an immediate early gene involved in regulating the process of information transfer within cells, can produce rapid and brief expression to the synaptic stimulation, and respond to changes in the excitability of nerve cells^[20]. Some studies suggested^[21–24], extraneous light stimulation on visual cortex cell membrane receptor and activate the second messenger in membrane and combine to the corresponding receptors, to induce the excitatory synaptic potentials, and cause the excitement of neurons. C-fos gene transcription is activated to translate into fos protein as a messenger, and further control the expression of late response genes. Other scholars put forward the competition mechanism of the visual cortex cell development and drift theory^[25], suggested that covering the healthy eye in a critical period of visual development can remove initiative inhibition in the cerebral cortex of health eye, after five months of visual deprivation, removing the contralateral eye can make visual cortex cells of deprived eye become sharply higher, indicating C-fos mRNA can reflect the functional status of the visual cortex cells. Visual developmental plasticity of critical period ends around 40–45 d after birth, fos proteins expression terminates

Table 1

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

Groups		P25	P35	P45	P120
Group A		17.58 \pm 4.25	11.87 \pm 5.68	5.98 \pm 4.90	5.59 \pm 5.68
Group B	Model eye	10.66 \pm 6.03	14.85 \pm 10.24	16.58 \pm 4.77	12.75 \pm 5.11
	Non-model eye	17.40 \pm 3.51	15.50 \pm 2.40	12.77 \pm 4.62	8.41 \pm 2.38
Group C	Model eye	14.62 \pm 5.88	16.47 \pm 4.97	15.09 \pm 4.83	11.36 \pm 2.97
	Non-model eye	12.18 \pm 5.88	14.94 \pm 7.02	16.79 \pm 4.02	11.36 \pm 5.37

Table 2

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

Groups		P25	P35	P45	P120
Group A		13.94 \pm 5.16	11.12 \pm 3.90	6.72 \pm 1.65	6.03 \pm 0.22
Group B	Model eye	13.27 \pm 6.21	8.79 \pm 1.44	8.23 \pm 1.80	8.99 \pm 3.10
	non-model eye	13.38 \pm 3.38	9.67 \pm 4.87	9.10 \pm 0.68	9.64 \pm 3.02
Group C	Model eye	14.53 \pm 2.68	7.99 \pm 2.69	9.48 \pm 1.85	9.22 \pm 4.37
	non-model eye	13.48 \pm 2.61	7.86 \pm 3.53	8.58 \pm 1.85	7.99 \pm 2.17

around the same period^[26,27]. In this study, groups B and C were monocular strabismus amblyopia model and visual deprivation amblyopia model, in the visual developmental plasticity in the critical period, including the early, late, and adult phases, expression of C-fos protein the lateral geniculate body and visual cortex was determined. Results showed that C-fos expression was hardly observed in the visual cortex of groups A and C. In groups B and C during the visual plasticity developmental critical late period and adult period, C-fos proteins were hardly seen in the first layer of visual cortex area 17, but were visible in the rest of the five layers, suggesting the visual cortex neurons exist a certain degree of visual plasticity. Based on the experimental results that, it is concluded that deprivation amblyopia in the key period of visual development can maintain the visual cortex in a state of primary or naïve, and prolong the visual developmental plasticity critical period. When receiving the light stimulation again, C-fos expression level in the visual cortex increases rapidly, can restart the neuronal plasticity mechanism.

According to the results of this study on deprivation and strabismus amblyopic visual rats, during late developmental stage and adult period, expression of C-fos protein in the lateral geniculate body and visual cortex neurons increased, suggesting that for adult amblyopia rats after sensitive period of visual development, the visual cortex neurons of rats still keep a certain degree of visual plasticity.

Conflict of interest statement

We declare that we have no conflict of interest.

References

- [1] An XY, Zhao X. Expression of PSD-95 in the visual cortex of the normal development and monocular deprivation rats. *J Ophthalmol* 2011; **31**(2): 127–128.
- [2] Sturm V. The lazy eye—contemporary strategies of amblyopia treatment. *Praxis* 2011; **100**(4): 229–235.
- [3] Delekate A, Zagrebelsky M, Kramer S, Schwab ME, Korte M. Nogo-A restricts synaptic plasticity in the adult hippocampus on a fast time scale. *Proc Natl Acad Sci USA* 2011; **108**(6): 2569–2574.
- [4] Lopez PH, Ahmad AS, Mehta NR, Toner M, Rowland EA, Zhang J. et al. Myelin-associated glycoprotein protects neurons from excitotoxicity. *J Neurochem* 2011; **116**(5): 900–902.
- [5] Sun XN, Tao J, Hao XG. Visual electrophysiology and visual cortex of monocular form-deprivation rats during sensitive period of visual development. *Ultrastructural Study* 2012; **12**(11): 2075.
- [6] Lee J, Liu XL, Mao Y. The expression of PSD-95 of the lateral geniculate body before and after sleep deprivation amblyopia rats with suture treatment in the key period. *Chin J Strabismus Pediatric Ophthalmol* 2013, **21**(3): 5–7.
- [7] Raiker SJ, Lee H, Baldwin KT, Duan Y, Shrager P, Giger RJ. Oligodendrocyte-myelin glycoprotein and Nogo negatively regulate activity-dependent synaptic plasticity. *J Neurosci* 2010; **30**(37): 12432–12445.
- [8] Schnaar RL, Lopez PH. Myelin-associated glycoprotein and its axonal receptor. *J Neurosci Res* 2009; **87**(15): 3267–3276.
- [9] Llorens F, Gil V, del Rio JA. Emerging functions of myelin-associated proteins during development, neuronal plasticity, and neurodegeneration. *FASEB J* 2011; **25**(2): 463–475.
- [10] Gibson DA, Ma L. Developmental regulation of axon branching in the vertebrate nervous system. *Development* 2011; **138**(2): 183–195.
- [11] Luo YL, Wu XY, Liu SZ. NEP1-40 visual cortex plasticity of adult monocular deprivation amblyopia rats reactivation. *Int J Ophthalmol* 2013; **13**(2): 246–251.
- [12] Li LL, Wen Y, Gong HQ. The function of graphic visual evoked potential of binocular vision in cats. *Int J Ophthalmol* 2013; **13**(5): 865–868.
- [13] Bastarriga G, Thilo C, Headden GF, Zwerner PL, Costello P, Schoepf UJ. Cardiac CT in the assessment of acute chest pain in the emergency department. *Am J Roentgenol* 2009; **193**(2): 397–409.
- [14] Zhang K, Zhang JJ, Liu Song T. Expression changes of the nerve growth factor of lateral geniculate body in deprived amblyopic rats. *J Med BBS Magazine* 2012; **33**(1): 17–19.
- [15] Lv YY, Zhao XL, Wang SZ, Hua Y. The expression of P53, Bax and Caspase 3 protein after traumatic optic nerve injury in rats. *J Tianjin Med* 2010; **38**(11): 996–998.
- [16] Zeng YW, He XZ, Wang Z. Expression of Caspase 3 in visual cortex of deprived amblyopia rats. *Int J Ophthalmol* 2012; **12**(11): 2069–2071.
- [17] D Bavelier, DM Levi, RW Li, Dan Y, Hensch TK. Removing brakes on adult brain plasticity: from molecular to behavioral interventions. *Neurosci* 2010; **30**(45): 14964–14971.
- [18] Harauzov A, Spolidoro M, DiCristo G, et al. Reducing intracortical inhibition in the adult visual cortex promotes ocular dominance plasticity. *J Neurosci* 2010, **30**(1): 361–371.
- [19] Sun XN, Zhang JS. Influences of levodopa on expression of N-methyl-D-aspartate receptor-1-subunit in the visual cortex of monocular deprivation rats. *Ophthalmol* 2012; **5**(1): 50–54.
- [20] Morishita H, Miwa JM, Heintz N, Hensch TK. Lynx1, a cholinergic brake, limits plasticity in adult visual cortex. *Science* 2010; **330**(6008): 1238–1240.
- [21] Silingardi D, Scali M, Belluomini G, Pizzorusso T. Epigenetic treatments of adult rats promote recovery from visual acuity deficits induced by long-term monocular deprivation. *Eur Neurosci* 2010; **31**(12): 2185–2192.
- [22] Fernando J, Vetencourt M, Baroncelli L, Tiraboschi E, Castren E, Cattaneo A, et al. IGF-1 restores visual cortex plasticity in adult life by reducing local GABA levels. *Neural Plast* 2012; **20**(12): 2504–2521.
- [23] Rashad MA. Pharmacological enhancement of treatment for amblyopia. *Clin Ophthalmol* 2012; **6**: 409–416.
- [24] Astle AT, McGraw PV, Webb BS. Can human amblyopia be treated in adult hood. *Strabismus* 2011; **19**(3): 99–109.
- [25] Sabel BA. Vision restoration after brain and retinal damage: the residual vision activation theory. *Prog Brain Res* 2011; **192**(1): 199–262.
- [26] Hess RF, Thompson B, Gole G, Mullen KT. Deficient responses from the lateral geniculate nucleus in humans with amblyopia. *Neurosci* 2009; **29**(5): 1064–1070.
- [27] Moore BD 4th, Kiley CW, Sun C, Usrey WM. Rapid plasticity of visual responses in the adult lateral geniculate nucleus. *Neuron* 2011; **71**(5): 812–819.