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Basic Study

Regulation of acupuncture on NMDAR1 mRNA expression in visual cortex of monocularly-deprived rats

针刺对单眼剥夺大鼠视皮层 NMDAR1 mRNA 表达调节的研究

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Abstract

Objective: To explore the molecular biological mechanism of acupuncture in intervening visual deprivation.

Methods: Forty-eight 2-week old Wistar rats were randomly divided into a normal group, a model group, and 6 acupuncture groups (group C1: acupuncture at the unaffected side in early stage; group C2: acupuncture at the affected side in early stage; group D1: acupuncture at the unaffected side in mid-stage; group D2: acupuncture at the affected side in mid-stage; group E1: acupuncture at the unaffected side in late stage; group E2: acupuncture at the affected side in late stage) by the random number table, 6 rats in each group. Rats in the normal group didn't receive any interventions. The rat model of deprivation amblyopia was established by unilateral eyelid suture in the model group and each acupuncture group. After successful modeling, rats in model group didn't receive any treatments; rats in the acupuncture groups received acupuncture intervention which began respectively on the 3rd, 12th and 21st day after modeling. Pattern visual evoked potential (P-VEP) and N-methy D-aspartatreceptor-1 (NMDAR1) mRNA expression in visual cortex area 17 were detected at the end of acupuncture intervention in each group.

Results: After the intervention, the P-VEP waveform was significantly changed, with a significantly delayed P_{100} value (P<0.01) and significantly decreased amplitude of N_{45}-P_{100} in the model group versus the normal group (P<0.01); the P-VEP waveform was significantly improved, with obviously earlier P_{100} (P<0.01) and increased amplitude of N_{45}-P_{100} (P<0.05) in each acupuncture group versus the model group. The improvement effect of acupuncture on the P-VEP waveform in group C1 and C2 was more significant than that in group D1, D2, E1 and E2. The expression of NMDAR1 mRNA of the rat visual cortex area 17 in the model group was significantly lower than that in the normal group (P<0.01); and the expression of NMDAR1 mRNA in the visual cortex area 17 of each acupuncture group was significantly higher than that in the model group (P<0.05); the effect of acupuncture on NMDAR1 mRNA expression in group C1 and C2 was more significant than that in group D1, D2, E1 and E2; and the effect of acupuncture on NMDAR1 mRNA expression was better in group C2 than in group C1 (P<0.05); there was no significant difference in the expression of NMDAR1 mRNA between group D1 and D2, neither between E1 and E2 (P>0.05).

Conclusion: P-VEP waveform is abnormal and NMDAR1 mRNA expression in visual cortex area 17 is decreased in rats with monocularly-deprived amblyopia. Acupuncture in the sensitive period can significantly regulate the abnormal P-VEP waveform and the down-regulate the NMDAR1 mRNA expression of the visual cortex of rats with monocularly-deprived amblyopia. Early treatment in the sensitive period should be the key to obtaining the curative effect.

Keywords: Acupuncture Therapy; Amblyopia; Receptors, N-Methyl-D-Aspartate; NMDA receptor A1; Evoked Potentials, Visual; Rats

【摘要】目的：探讨针刺干预视觉剥夺效应的分子生物学机制。**方法：**采用随机数字表法将 48 只 2 周龄的 Wistar 大鼠随机分为正常组、模型组和 6 个针刺组[早期针刺患侧组(C1)、早期针刺健侧组(C2)、中期针刺患侧组(D1)、中期针刺健侧组(D2)、晚期针刺患侧组(E1)和晚期针刺健侧组(E2)]，每组 6 只。正常组不予任何处理；模型组和各针刺组采用单侧眼睑缝合的方法建立剥夺性弱视动物模型，造模成功后，模型组不予任何治疗；早期、中期、晚期针刺组分别于造模后第 3 天、12 天、21 天开始针刺治疗。治疗结束后检测各组大鼠图形视觉诱发电位(P-VEP)和视皮层 17 区 N-甲基-D-天门冬氨酸受体 1(NMDAR1) mRNA 的表达。**结果：**模型组大鼠 P-VEP 波形较正常组有明显改变，表现为 P_{100} 时值明显延迟(P＜0.01)，N_{45}-P_{100} 幅值显著降低(P<0.01)；治疗后，各针刺组大鼠 P-VEP 波形较

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模型组均有显著改善，表现为 P_{100} 时值明显提前($P<0.01$)，N_{45}-P_{100} 幅值明显升高($P<0.05$)；并且早期针刺对 P-VEP 波形的影响大于中期和晚期针刺。模型组大鼠视皮层 17 区 NMDAR1 mRNA 的表达较正常组明显降低($P<0.01$)；而治疗后各针刺组大鼠视皮层 17 区 NMDAR1 mRNA 表达较模型组均显著提高($P<0.05$)；其中早期针刺对 NMDAR1 mRNA 表达水平的影响大于中期和晚期针刺；而早期针刺患侧穴位对 NMDAR1 mRNA 表达水平的影响优于针刺健侧穴位($P<0.05$)；中期、晚期针刺患侧穴位与健侧穴位对 NMDAR1 mRNA 表达水平的影响差异无统计学意义($P>0.05$)。**结论：**单眼剥夺后大鼠 P-VEP 波形出现异常改变，视皮层 17 区 NMDAR1 mRNA 表达减少。敏感期内针刺对单眼剥夺大鼠异常的 P-VEP 波形和视皮层 17 区 NMDAR1 mRNA 低水平表达均具有明显的调节作用，并且敏感期内早期治疗是取得疗效的关键。

【关键词】针刺疗法；弱视；受体; N-甲基-D-天门冬氨酸; N-甲基-D-天门冬氨酸受体 A1；诱发电位，视觉；大鼠
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Amblyopia is a common eye problem in clinic that impairs children's visual development. The incidence of amblyopia in China is 3.0% to 3.8%, and children account for 25.0% of the whole affected population. Amblyopia seriously affects the healthy development of visual function of children in China[1]. Acupuncture is a safe, fast and effective therapy for amblyopia. Therefore, it has been widely used in clinical practice[2-3]. Our previous work showed that acupuncture therapy had significant efficacy on amblyopia, and could effectively antagonize the deprivation effect on the visual development in the sensitive period. The mechanism may be related to promoting the brain-derived neurotrophic factor, brain derived neurophic factor (BDNF) and N-methyl-D-aspartate receptor (NMDAR) synthesis, secretion and gene expression in visual system[4]. In this study, visual electrophysiology and reverse transcription polymerase chain reaction (RT-PCR) were used to investigate the effect of acupuncture, based on years of clinical experience in acupoints selection, by focusing on the mRNA expression of visual pattern visual evoked potential (P-VEP) and NMDAR1 (synaptic development-related receptors of visual cortex neurons). The possible molecular mechanism of acupuncture for monocularly-deprived amblyopia in rat models in the sensitive period was explored.

1 Materials and Methods

1.1 Animals and grouping

A total of 48 Wistar rats, 2-week old, were provided by the Experimental Research Center of Gansu University of Traditional Chinese Medicine.

All rats were randomly divided into a normal group, a model group, group C1 (acupuncture at the unaffected side in early stage), group C2 (acupuncture at the affected side in early stage), group D1 (acupuncture at the unaffected side in mid-stage), group D2 (acupuncture at the affected side in mid-stage), group E1 (acupuncture at the unaffected side in late stage), and group E2 (acupuncture at the affected side in late stage) by the random number table method, 6 rats in each group.

1.2 Experimental apparatuses

Multifunction pattern reversal visual evoked potential (PRVEP) instrument (Gale Incorporation, USA), TC-512 gradient gene amplifier (Techne Incorporation, UK), SmartSpec 3000 spectrophotometer (Bio-Rad Laboratories, USA), horizontal electrophoresis system (Beijing 61 Instrument Factory, China), PowerPac basic electrophoresis apparatus (Bio-Rad Laboratories, USA), TANNON-2010 digital gel image analysis system and GIS 1D image analysis software (Beijing Yuanpinghao Biotech Co., Ltd., China), −80 ℃ freezer (Thermo Fisher Scientific, USA), −20 ℃ freezer (Haier Company, China), low temperature high speed centrifuge (Beckman Coulter, USA), mini high-speed centrifuge (Eppendorf, Germany), constant temperature water bath box (Harbin Dongfang Electronic Control Switch Factory, China), V-1000 type vortex oscillator (Taipei Rilong Instrument Co., Ltd., China), ultra-pure water system (Membrapure Company, Germany), P20, P100 and P1000 precision pipette (Gilson Company, USA), double copper mesh shield (Anhui Suixi Zhenghua Teaching & Testing Instrument Factory, China), myoelectricity evoking potentiometer (Guangzhou SUNJAVA Medical Information Industry Co., Ltd., China), electronics balance (Sartorius, Germany).

1.3 Experimental reagents and drugs

Urethane (batch number: 635810, Sigma-Aldrich, USA), TRIZOL (batch number: 15596-018, Invitrogen, USA), diethylpyrocarbonate (DEPC, batch number: 1609-47-8, Sigma, USA), RT-PCR kit (batch number: FP202-02, Promega, USA), absolute ethyl alcohol (batch number: 20070620, Beijing Chemical Plant, China), isopropyl alcohol (IPA, Batch number: 110313, Tianjin Fuyu Fine Chemical Co., Ltd., China), trichloromethane (batch number: 67-66-3, Beijing Chemical Factory, China), agarose (batch number: 111760, Biowest Company, France), ethidium bromide (EB, batch number: BC100331, Changchun Xinhuayu Biotechnology Company, China), trihydroxymethylaminomethane (THAM, batch number: 77-86-1, Shanghai Boyao Biotechnology Co., Ltd., China), orthoboricacid (batch number: 20070223, Beijing Chemical Factory, China), ethylene diamine tetraaceticacid-2Na (EDTA-2Na, batch number: XF13-20100520, Tianjin Guangfu Technology Development Co., Ltd., China), 0.9% NaCl (batch number: S11040202, Changchun Haobang Pharmaceutical Co., Ltd., China), hydrochloric acid

(batch number: XK13-201-00416B, Zhuhai Huachengda Chemical Co., Ltd., China), DNA marker (batch number: DM080530, Changchun Baotaike Biology Co., Ltd., China), glycerol (batch number: 20061204, Beijing Chemical Factory, China), primers (Dalian Bio-engineering Co., Ltd., China).

2 Methods

2.1 Model preparation

For the experimental rats, the left or right eye was randomly selected to establish the monocular visual deprivation model according to the literature[5]. Rats in the model group and each acupuncture group were given 20% urethane [0.5 mL/(kg·bw)] intraperitoneally for anesthesia. After the hair around the eyelid margin was shaved, 0.5-1 mm eyelid edges (both upper and lower) were cut from the inner canthus to the outer canthus. Subcutaneously, the skin was sutured to close the experimental eye for establishing the monocular deprivation (MD) rat models. Antibiotics were routinely used after the operation.

2.2 Treatment in different groups

2.2.1 Normal group

Rats in the normal group were routinely reared without any interventions. At the end of the experiment, P-VEP was detected. The rat's brain was isolated and the expression of NMDAR1 mRNA was detected.

2.2.2 Model group

Rats in the model group did not receive any interventions after successful modeling. At the end of the experiment, the experimental eyelids were opened and the P-VEP of the experimental eye was measured after the cornea had been thoroughly exposed for 30 min. Then the rat's brain was isolated and the expression of NMDAR1 mRNA was detected.

2.2.3 Acupuncture groups

Rats in the 6 acupuncture groups received the acupuncture intervention accordingly after modeling.

Acupoints: Jingming (BL 1), Cuanzhu (BL 2), Guangming (GB 37) and Fengchi (GB 20).

Methods: The acupoints were located according to the *Experimental Acupuncture Science*[6].

Acupuncture was performed with acupuncture needles of 0.30 mm in diameter and 13 mm in length. Acupuncture in group C1 and C2 started on the 3rd day after the modeling; acupuncture in group D1 and D2 started on the 12th day after the modeling; acupuncture in group E1 and E2 began on the 21st day after the modeling. Jingming (BL 1), Guangming (GB 37) and Fengchi (GB 20) were perpendicularly punctured for 2-3 mm, and Cuanzhu (BL 2) was obliquely punctured for 2-3 mm. Even reinforcing-reducing manipulations were used and the acupuncture was performed once daily, for 10 min/time. The experimental eyelids of rats in each acupuncture group were opened at the end of the acupuncture intervention. The P-VEP of the experimental eye was detected after the cornea had been fully exposed for 30 min.

3 Observed Items

3.1 P-VEP detection

3.1.1 P-VEP detection procedures

Rats were weighed and anesthetized by intraperitoneal injection of chloral hydrate [4%, 2 mL/(kg·bw)]. Two drops of tropicamide and phenylbutazone were used for each eye respectively, to cause corectasis and contraction of nictitating membrane and eyelid membrane. The rats were immobilized during the detection. The binocular visual axis was adjusted to make the corneal light reflection point and the screen center parallel, and then the rats were placed inside the electrostatic shield for test.

3.1.2 Standards for P-VEP testing

Reversal stimulator settings: Stimulus frequency at 4 Hz; ideograph of checkerboard; graphics size of 8×6 with full field of vision.

Amplifier settings: Upper limit frequency of 500 Hz, lower limit frequency of 1 Hz; recording electrode of Oz, reference electrode of FPz; sampling time of 300 ms; superposition frequency of 128 times; impedance between recording electrode and reference electrode of less than or equal to 5 Ω.

A stainless steel needle electrode was used. The recording electrode was placed at 1.5 cm above the occipital tuberosity. The reference electrode was inserted into the glabellum of the forehead. The vertical distance between the cornea and the visual stimulus screen was 57 cm.

3.2 Amplification and expression detection of NMDAR1 mRNA in visual cortex

3.2.1 Tissue sample preparation

The rats were sacrificed by cervical dislocation. The visual cortex area 17 was isolated, put into Eppendorf (EP) tubes, and then quickly transferred to liquid nitrogen and stored in a refrigerator at –80 ℃.

3.2.2 Extraction of total RNA from visual cortex

Total RNA was extracted from the brain visual cortex using TRIZOL reagent and detected by RT-PCR.

Detailed steps: A total of 1 mL TRIZOL was added into 100 mg fresh tissue to prepare the homogenate; reversed 10 times for mixing, incubated at room temperature for 5 min and then added 1/5 volume chloroform (0.2 mL); the mixture was centrifuged at 120 000 r/min for 15 min at 4 ℃, about 400 μL of the upper aqueous phase was transferred into another 1 mL EP tube. Added an equal volume of isopropanol (about 400 μL) and mixed, then incubated at room temperature for 10 min; then centrifuged at

120 000 r/min for 10 min; discarded the supernatant, and added 1 mL pre-cooled 75% ethanol (prepared with DEPC water); then centrifuged at 7 500 r/min for 5 min at 4 ℃ and the supernatant was discarded. Air drying for 5-10 min and dissolved in 20 μL DEPC solution; added 1 μL RNA safe and incubated at 60 ℃ water bath for 20 min, transferred into −80 ℃ refrigerator when it was cooled.

3.2.3 RNA purity and concentration determination

RNA concentration was detected by ultraviolet spectrophotometer at A260 and A280, data was kept if the value of A260/A280 was between 1.7 and 2.0, otherwise was discarded. RNA concentration was calculated after the total RNA was diluted for 40 folds. The volume of total RNA was adjusted according to the RNA concentration, so that the total RNA amount of each sample was the same in the reverse transcription system.

3.2.4 NMDAR1 mRNA synthesis, amplification and expression detection

Primer sequences: Primer sequences were designed according to the reference[7], and synthesized by Dalian Takara Biological Co., Ltd. (Table 1).

Table 1. Primer sequences for each gene in the PCR reaction

Primer name	Sequence (from 5' to 3')
NMDAR1	TGGCCGATTCAAGGTGAACA (upstream)
	CCATGCCTAGGATACGTGCAGA (downstream)
β-actin	GCTTCTTTGCAGCTCCTTCGT (upstream)
	ATATCGTCATCCATGGCGAAC (downstream)

RT-PCR was performed for the target gene NMDAR1 and the internal control gene β-actin in the same system, and then the products were subjected to agarose gel electrophoresis. The relative density ratio of NMDAR1 and β-actin gene bands in the same lane was used as the response of NMDAR1 mRNA expression levels.

Establishment of one-step RT-PCR reaction system: The total RNA volume was adjusted according to the measured RNA concentration, so that the total RNA amount of each sample was the same in the reverse transcription system. The following reaction system was established after multiple pretests with gradient conditions (Table 2).

One-step RT-PCR reaction program: A cycle of reverse transcription (48 ℃, 45 min; 94 ℃, 2 min). Forty cycles of degeneration, annealing and extension (94 ℃, 30 s; 59.2 ℃, 1 min; 68 ℃, 2 min). A cycle of final extension (68 ℃, 7 min, 4 ℃, end reaction).

After the amplification, 3 μL of the RT-PCR product was mixed with 1 μL of the loading buffer, and then 3 μL of the mixture was electrophoresed on a 1.5% agarose gel with EB at 60 V for 100 min.

Table 2. RT-PCR reaction system

Reagent	Volume
NMDAR1 upstream primer	0.5 μL
NMDAR1 downstream primer	0.5 μL
β-actin upstream primer	0.5 μL
β-actin downstream primer	0.5 μL
AMV Buffer	10 μL
dNTP	2 μL
$MgSO_4$	4 μL
AMV RT	1 μL
Tfl	1 μL
RNA	x μL (containing2000 ng total RNA)
No nuclear enzyme water	30–x μL
Total reaction system	50 μL

4 Experimental Results

4.1 P-VEP test results

Compared with the normal group, the P_{100} of P-VEP waveform in the model group was significantly delayed, and the amplitude of N_{45}-P_{100} was significantly decreased. The difference was significant ($P<0.01$), indicating that the MD model was successfully established. Compared with the model group, the P_{100} was significantly earlier and the amplitude of N_{45}-P_{100} was significantly higher in the acupuncture groups ($P<0.01$). The difference was significant, indicating that acupuncture could antagonize the visual deprivation; there was no significant difference between the two early-stage acupuncture groups and the normal group ($P>0.05$). The differences between the early-stage acupuncture groups and the mid-stage acupuncture groups were statistically significant ($P<0.05$), indicating that acupuncture in the early stage should be better than that in the mid-stage. The differences between the late-stage acupuncture groups and the mid-stage acupuncture groups were statistically significant ($P<0.05$), indicating that acupuncture in the mid-stage should be better than that in the late stage (Table 3).

4.2 The expression of NMDAR1 mRNA in visual cortex area 17

The total RNA from rat's visual cortex was amplified by RT-PCR. The PCR products were subjected to agarose gel electrophoresis, and then the images were obtained and analyzed using GIS digital gel imaging system. It was found that the NMDAR1 mRNA expression level in the visual cortex area 17 of rats was different among the groups. In the current study, the NMDAR1 mRNA expression level in each group was presented as the relative density ratio between the NMDAR1 and β-actin gene bands (Figure 1).

Table 3. Comparing rat P-VEP among groups ($\bar{x}$ ±*s*)

Group	*n*	P_{100} (ms)	N_{45}-P_{100} (μv)
Normal group	6	25.25±3.17	20.92±5.69
Model group	6	45.79±6.38[1)]	8.02±2.10[1)]
Group C1 and C2	12	26.12±2.58[2)3)]	18.96±5.15[2)3)]
Group D1 and D2	12	32.94±5.01[2)4)]	15.31±4.05[2)4)]
Group E1 and E2	12	39.16±5.01[2)]	11.68±4.18[2)]

Note: Compared with the normal group, 1) *P*<0.01; compared with the model group, 2) *P*<0.01; compared with group D1 and D2, 3) *P*<0.05; compared with group E1 and E2, 4) *P*<0.05

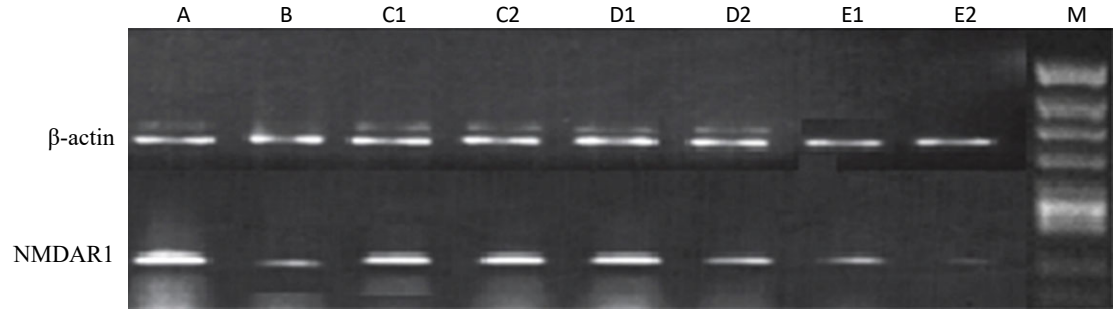

Figure 1. NMDAR1 mRNA expression in visual cortex area 17 in each group

Note: A=Normal group; B=Model group; C1=Group C1; C2=Group C2; D1=Group D1; D2=Group D2; E1=Group E1; E2=Group E2; M=Marker

Compared with the normal group, the expression of NMDAR1 mRNA of the visual cortex area 17 in the model group was significantly decreased, and the difference was statistically significant ($P < 0.01$), suggesting that MD had a significant effect on NMDAR1 mRNA expression in rat's visual cortex; compared with the model group, the expression of NMDAR1 mRNA in rat's visual cortex significantly increased in the acupuncture groups ($P<0.01$, $P<0.05$), suggesting that acupuncture intervention could improve the NMDAR1 mRNA expression of the visual deprivation rats and the deprivation influence; the expression of NMDAR1 mRNA in the visual cortex area 17 in group C2 was insignificantly different from that in the normal group ($P > 0.05$), but the expression in group C1 was significantly different from that in the normal group ($P<0.05$), indicating that early-stage acupuncture at the affected side could significantly increase the NMDAR1 mRNA expression in the visual cortex area 17, approaching the normal level; the differences between the early-stage acupuncture groups and the mid-stage acupuncture groups were statistically significant ($P < 0.05$), indicating that the effect of early-stage acupuncture on the NMDAR1 mRNA expression in visual cortex area 17 was more significant; the differences between the late-stage acupuncture groups and the mid-stage acupuncture groups were statistically significant ($P < 0.05$), indicating that the effect of mid-stage acupuncture on the NMDAR1 mRNA expression in visual cortex area 17 was more significant; there was a statistically significant difference in the expression of NMDAR1 mRNA between group C1 and C2 ($P < 0.05$), indicating that acupuncture at the affected side showed a better effect on the expression of NMDAR1 mRNA in visual cortex area 17 in the early stage of MD; there were no statistically significant differences in the expression of NMDAR1 mRNA between group D1 and D2, neither between group E1 and E2 ($P>0.05$), indicating that in the mid-stage and late stage of MD, acupuncture at the affected and unaffected sides produced parallel effects on the expression of NMDAR1 mRNA in visual cortex area 17 (Table 4).

Table 4. Expression levels of NMDAR1 mRNA in visual cortex 17 area ($\bar{x}$ ±*s*)

Group	*n*	NMDAR1/β-actin
Normal group	6	0.88±0.02
Model group	6	0.43±0.07[1)]
Group C1	6	0.81±0.04[2)3)6)]
Group C2	6	0.84±0.05[3)5)7)]
Group D1	6	0.68±0.05[3)8)]
Group D2	6	0.70±0.03[3)9)]
Group E1	6	0.50±0.04[4)]
Group E2	6	0.53±0.03[4)]

Note: Compared with the normal group, 1) *P*<0.01; 2) *P*<0.05; compared with the model group, 3) *P*<0.01, 4) *P*<0.05; compared with group C1, 5) *P*<0.05; compared with group D1, 6) *P*<0.05; compared with group D2, 7) *P*<0.05; compared with group E1, 8) *P*<0.05; compared with group E2, 9) *P*<0.05

5 Discussion

In this study, Wistar rats (2-week old) underwent eyelid suture to establish rat models of MD amblyopia. The molecular mechanism of acupuncture in intervening visual deprivation was explored by detecting the rat's P-VEP and NMDAR1 mRNA expression in visual cortex before and after acupuncture, based on the visual cortical neuron development.

P-VEP is the electrical activity evoked by visual transmission to the visual cortex in the occipital lobe, when eyes are stimulated by the black-and-white image reversal and recorded in the cerebral cortex. It reflects the functional state of the retinal ganglion cells to the visual cortex. It is one of the important ways to check the visual damage. In general, P-wave amplitude reflects the patient's visual acuity, while it reflects the state of the optic nerve conduction during the latent period[8]. The latent time of the P_1 wave will be shortened gradually, while the amplitude of N_1-P_1 wave will gradually become larger, if the visual development is normal[9]. The latent time of N wave and P wave is extended, and the P_{100} peak amplitude is decreased in P-VEP of the amblyopic eye, compared with that of the normal eye[10]. The results in this study showed that the P-VEP waveform of rats in the model group appeared abnormal after 4 weeks of the MD model establishment, with significantly delayed P_{100} and significantly decreased amplitude of N_{45}-P_{100}, indicating that MD model was successfully established. Compared with the model group, the appearance time and amplitude of rats in the acupuncture groups were significantly changed after acupuncture intervention. P_{100} of the P-VEP waveform appeared earlier and amplitude of the N_{45}-P_{100} increased obviously. These results showed that acupuncture therapy can antagonize the damage of visual deprivation in the visual system during the sensitive period of visual development. Acupuncture in the early stage had a better effect on P-VEP waveform than that in the middle and late stages. This further confirmed the antagonistic effect of acupuncture on visual deprivation. The earlier the acupuncture intervention, the more obvious effect on P-VEP during the sensitive period of visual development, indicating the strongest visual plasticity during the early sensitive period of visual development and the critical period for clinical treatment.

NMDAR is a subtype of ionotropic glutamate receptors. Functional NMDARs must contain subunit 1 (NR1). Multiple subunits 2 (NR2) and NR1 form a tetramer (or pentamer), which plays a biological role in neurons survival, dendritic and axonal structure development, synaptic plasticity and regeneration, thus regulating the development of the central nervous system during the brain development. This is achieved by selective expression of different receptor subtypes and changes in their structure and function, therefore, affecting the receptor-mediated Ca^{2+} influx and regulating the second messenger system[11]. NR1 is widely distributed in neurons of the central nervous system. It is closely related to the survival of neurons, development of visual system and synaptic plasticity[12]. Therefore, the abnormal response of NMDAR1 can cause central nervous system dysfunction. Our results showed that during and at the end of the sensitive period of vision deprivation, the activity and number of NMDAR1 in visual cortex area 17 were reduced[13]. Yin ZQ, *et al*[14] also found that the NMDAR1 expression in each layer of visual cortex was reduced 3 weeks after the strabismus surgery, which started from 1 week after the strabismus surgery, by investigating the NMDAR1 expression in the visual cortex of the strabismic amblyopia cats. The results of our current study showed that NMDAR1 mRNA expression in visual cortex area 17 of rats with monocular vision deprivation was significantly reduced, which was consistent with the reports. Contemporary studies suggested that acupuncture can stimulate the central nervous system to improve the nerve function of the visual cortex.

Current studies showed that, after nerve injury, an important mechanism that acupuncture and moxibustion promoted the nerve repair mainly depending on nerve regeneration. Promoting optic nerve regeneration has become an important basis for acupuncture to facilitate the repair of optic nerve injury[15-20]. The results in this study showed that monocular vision deprivation can cause molecular biological changes in rat's visual cortex area 17, which was evidenced by significantly reduced NMDAR1 mRNA expression.

The visual deprivation effect was significantly improved by acupuncture with significantly increased NMDAR1 mRNA expression level in visual cortex area 17, indicating that acupuncture intervention can improve visual deprivation; early-stage acupuncture showed the most significant effect on up-regulating the NMDAR1 mRNA expression after monocular vision deprivation, which was better than the mid-stage and late-stage acupuncture. This indicated that, during the visual development, acupuncture in the early stage of the sensitive period can produce the strongest plasticity effect. Acupuncture at the affected side was better than that at the unaffected side in improving vision deprivation in the early stage after MD; acupuncture at the affected side and the unaffected produced parallel effects in improving vision deprivation in the middle and late stages after MD.

In the present study, only the expression of NMDAR1 mRNA in the visual cortex area 17 of the MD rats was compared and analyzed. The expression of NMDAR1 mRNA in other areas of the visual cortex was not detected. Whether the changes of P-VEP and NMDAR1

mRNA expression are involved in the rest areas still need further studies in the future.

Conflict of Interest

There was no potential conflict of interest in this article.

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Statement of Human and Animal Rights

The treatment of animals in this experiment conformed to the ethical criteria.

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