NEURAL REGENERATION RESEARCH
Volume 8, Issue 26, September 2013

www.nrronline.org

doi:10.3969/j.issn.1673-5374.2013.26.002 [http://www.nrronline.org; http://www.sjzsyj.org]
Ma GL, Song TB, Chen M, Fu Y, Xu Y, Ma ES, Wang W, Du J, Huang MX. Hippocampal and thalamic neuronal metabolism in a putative rat model of schizophrenia. Neural Regen Res. 2013;8(26):2415-2423.

Hippocampal and thalamic neuronal metabolism in a putative rat model of schizophrenia

Guolin Ma[1], Tianbin Song[2], Min Chen[3], Yuan Fu[3], Yong Xu[4], Ensen Ma[1], Wu Wang[1] Jiang Du[5], Mingxiong Huang[6]

1 *Department of Radiology, China-Japan Friendship Hospital, Beijing 100029, China*
2 *Department of Radiology, Beijing Shunyi Hospital, Beijing 101300, China*
3 *Department of Radiology, Beijing Hospital Affiliated to the Ministry of Public Health, Beijing 100730, China*
4 *Department of Mental Health, First Hospital of Shanxi Medical University, Taiyuan 030001, Shanxi Province, China*
5 *Department of Radiology, University of California, San Diego, CA 92103-8226, USA*
6 *Radiology Imaging Laboratory, Department of Radiology, University of California, San Diego, CA 92121, USA*

Research Highlights

(1) This study is the first to report MRI of early growth response protein 3 gene (*Egr3*) transfected rats as a putative model of schizophrenia.
(2) 3.0 T proton magnetic resonance spectroscopy of *in vivo* brain tissues showed metabolic abnormalities in hippocampal and thalamic neurons of growth response protein 3 transfected rats.
(3) This study revealed characteristics of proton magnetic resonance spectroscopy of the campus and thalamus of *Egr3* transfected rats and provided imaging evidence that may be useful in the early diagnosis and pathogenesis of schizophrenia.

Abstract

The transcription factor early growth response protein 3 (EGR3) is involved in schizophrenia. We developed a putative rat model of schizophrenia by transfecting lentiviral particles carrying the *Egr3* gene into bilateral hippocampal dentate gyrus. We assessed spatial working memory using the Morris water maze test, and neuronal metabolite levels in bilateral hippocampus and thalamus were determined by 3.0 T proton magnetic resonance spectroscopy. Choline content was significantly greater in the hippocampus after transfection, while N-acetylaspartate and the ratio of N-acetylaspartate to creatine/phosphocreatine in the thalamus were lower than in controls. This study is the first to report evaluation of brain metabolites using 3.0 T proton magnetic resonance spectroscopy in rats transfected with *Egr3*, and reveals metabolic abnormalities in the hippocampus and thalamus in this putative model of schizophrenia.

Key Words

neural regeneration; neuroimaging; schizophrenia; proton magnetic resonance spectroscopy; early growth response protein 3; hippocampus; thalamus; gene; neuroregeneration

Guolin Ma, M.D., Chief physician, Associate professor.

Corresponding author: Min Chen, M.D., Chief physician, Professor, Doctoral supervisor, Department of Radiology, Beijing Hospital Affiliated to the Ministry of Public Health, Beijing 100730, China, Chenmin62@yahoo.com.

Received: 2013-04-18
Accepted: 2013-07-01
(N20120820002)

Acknowledgments: We thank Song X, Shanxi Medical University in China, for help in animal experiments and data processing.

Author contributions: Ma GL conceived and designed the study, provided and integrated experimental data, analyzed the data, and wrote the manuscript. Song TB integrated and analyzed the data and conducted statistical analysis. Chen M guided the study and revised the manuscript. Wang W and Ma ES analyzed the experimental data. Fu Y conducted MRI scanning. Xu Y established animal models and analyzed the experimental data. Du J and Huang MX revised the manuscript. All authors approved the final version of the manuscript.

INTRODUCTION

Schizophrenia is a prevalent mental disorder, clinically characterized by uncoordinated thought, emotion and behaviors, paranoid or bizarre delusions, or disorganized speech and thinking, and it is accompanied by significant social dysfunction[1]. Its complex pathogenesis remains poorly understood; hypotheses include disorders of neurotransmitters, neurodevelopment, or neuronal membrane phospholipid metabolism[2]. Studies suggest that, depending on the gene,

Conflicts of interest: None declared.

Ethical approval: This study received permission from the Animal Ethics Committee of China-Japan Friendship Hospital, China.

Author statements: The manuscript is original, has not been submitted to nor is under consideration by another publication, has not been previously published in any language or any form, including electronic, and contains no disclosure of confidential information or authorship/patent application disputations.

the rate of the heredity susceptibility for schizophrenia is 65–85%[3].

The transcription factor early growth response factor (EGR)-3 plays an important role in cell proliferation, and muscular and neural development[4]. EGR3 can regulate a variety of signaling pathways, including nerve growth factor (NGF)-, brain-derived neurotrophic factor (BDNF)- and neuregulin 1 (NRG1)-mediated conduction pathways[5-8]. BDNF and NRG1 are important genes and signaling pathways that are altered in schizophrenia. EGR1 and EGR2 are induced by BDNF in primary cortical neurons[9]. EGR3 is the target gene of EGR1[10]. EGR3 and EGR1 can directly regulate the expression of the nerve growth factor receptor $p75^{NTR}$[11], and both *p75NTR* and *EGR3* are involved in axonal extension[12].

With a deepening understanding of *EGR3*-regulated signaling pathway, one phenomenon has aroused attention. The calcineurin/nuclear factor of activated T cells signaling pathway is essential for neuronal development and axonal growth, but is minimally or not at all involved in neuronal survival[13-14]. Likewise, *EGR3* is also required for axonal extension and branching but is not necessary for neuronal survival[5]. Furthermore, NGF and BDNF can activate nuclear translocation of the nuclear factor of activated T cells (NFAT) and the NFAT-dependent transcription in cortical neurons[14]. NFAT can directly trigger the transcription of *EGR2* and *EGR3*, and *EGR3* transcription subsequently triggers cell apoptosis through the activation of Fas ligand expression[15]. The above results indicate that *EGR3* may be the key regulatory factor in the calcineurin/NFAT signaling pathway. In addition, the *PPP3CC* gene, which encodes the calcineurin γ subunit, and *EGR3* are located adjacent to each other on chromosome 8. Both genes have been reported to associate with schizophrenia[16-18] and the two proteins are abnormally expressed in the brains of schizophrenic patients[17]. Furthermore, *Egr3* knockout mice are resistant to the adverse effects of antipsychotic drugs[19], similarly to patients with schizophrenia. According to the neurodevelopmental disorder hypothesis, *EGR3* expression regulates the calcineurin/NFAT signaling pathway, and dysfunction of this pathway induces schizophrenia. EGR3, located at chromosome 8p21.3, has been proposed as a susceptibility gene for schizophrenia[20].

Animal models of schizophrenia can be induced by drugs, brain injury and environmental changes, or genetic modification. Using transgenic technology, it is possible to transfect schizophrenia pathogenesis-related genes into animals to induce a schizophrenia-like phenotype. To our knowledge, this is the first study to report a model of schizophrenia using *Egr3* transfected rats.

Risperidone shows better clinical effects compared with traditional antipsychotics[21]. We assessed changes in escape latency and working memory in *Egr3*-transfected mice with or without risperidone treatment to determine whether this antipsychotic reversed the effects of the gene transfection.

Proton magnetic resonance spectroscopy is a non-invasive, non-radioactive method of detecting metabolites in certain brain regions using magnetic resonance and chemical shift techniques. It is currently the only method of detecting cellular metabolism *in vivo*. Proton magnetic resonance spectroscopy can reveal abnormal metabolites and abnormal changes in metabolites. Previous proton magnetic resonance spectroscopy showed metabolic abnormalities in the prefrontal lobe, hippocampus, cingulate gyrus and thalamus in patients with schizophrenia[22-23]. In addition, hippocampal injury plays a key role in the incidence and progression of schizophrenia, and the anterior hippocampal volume of patients with schizophrenia is smaller than that in the healthy population[24]. The thalamus has a major role in the experience and expression of emotions. N-acetylaspartate and the ratio of N-acetylaspartate to creatine are reduced in the thalamus of patients with schizophrenia[25]. We therefore selected the hippocampus and thalamus as regions of interest in the present study. We developed a putative

rat model of schizophrenia by transfection of *Egr3* and examined neuronal me tabolism and the model *in vivo* using proton magnetic resonance spectroscopy. Early proton magnetic resonance spectroscopy of rat brain was limited to tissue analysis *in vitro*, while recent *in vivo* studies have frequently used magnetic field strength > 4.0 T, which limits its use in rats. However, MRI with a magnetic field strength of 3.0 T enables multivoxel proton magnetic resonance spectroscopy in brain tissues of rat models of schizophrenia *in vivo*. To date, no studies have reported magnetic resonance spectroscopy in a rat model of schizophrenia induced by *Egr3* transfection. Selection of regions of interest is critical in magnetic resonance spectroscopy. Single voxel proton magnetic resonance spectroscopy has often been used to date; however, the cerebrospinal fluid, fat and other substances around the regions of interest can influence the results[25]. The present study used multivoxel proton magnetic resonance spectroscopy, which can collect data from multiple voxels at once. The voxel position of an appropriate region of interest is selected, which is much smaller than that defined in single voxel proton magnetic resonance spectroscopy. Thus, this method reduces the partial volume effect, and increases the reliability of results. In addition, shimming (elimination of field inhomogeneities) is optimized, and an appropriate saturation zone is placed[26-27]. Based on preliminary experiments, 10 zones of saturation were added to the regions of interest of the scout image, to reduce interference around the regions of interest and improve the quality of images.

While previous studies using proton magnetic resonance spectroscopy have focused on patients with schizophrenia, the biochemical alterations in the disorder require further examination in different brain regions and at different stages of disease progression. The use of animal models can often provide this information. We used proton magnetic resonance spectroscopy to study neuronal metabolite content in rats transfected with the *Egr3* gene as a putative model of schizophrenia.

RESULTS

Quantitative analysis of experimental animals

Twenty-four rats were randomly assigned to four groups: a schizophrenia model group (schizophrenia group), in which lentiviral particles carrying *Egr3* were injected bilaterally into the hippocampus and dentate gyrus; a second model group that additionally received intraperitoneal injections of risperidone for 2 weeks (risperidone group); a sham-operated group, in which lentiviral particles carrying green fluorescent protein were injected bilaterally into the hippocampus and dentate gyrus; and a control group that received intraperitoneal injections of normal saline only. There were six animals in each group and all 24 rats were included in the final analysis.

Behavioral characterization of *Egr3*-transfected rats as a model of schizophrenia

A Morris water maze working memory test was conducted 2 days after the final injection of risperidone or saline. The escape latency was significantly prolonged in rats transfected with the *Egr3* gene compared with control and sham-surgery groups ($P < 0.05$), indicating that *Egr3* transfection causes an impairment in working memory. Risperidone treatment reversed the above changes (Figure 1). This indicates that *Egr3*-transfected rats have clinically relevant features of schizophrenia, demonstrating that the model may be useful in the study of cognitive features of the disorder.

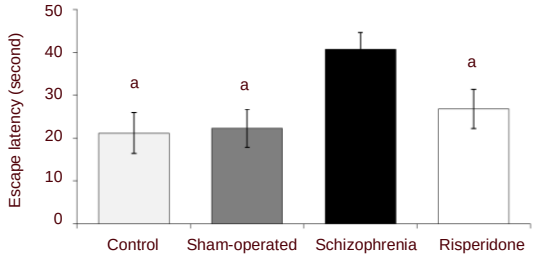

Figure 1 Impaired working memory in schizophrenia model rats in the Morris water maze test.

The time taken for the rat to find the platform was recorded. The escape latency was significantly prolonged in rats transfected with the *Egr3* gene ($P < 0.05$), indicating an impairment in working memory and reflecting a core feature of schizophrenia. The impairment was reversed after risperidone treatment, further supporting the *Egr3*-transfected rat as a novel model for schizophrenia. Data are expressed as mean ± SD of six rats in each group (one-way analysis of variance and Fisher's least significant difference test *post-hoc* test). $^{a}P < 0.05$, *vs*. schizophrenia group. *Egr3*: Early growth response protein 3 gene.

Proton magnetic resonance spectroscopy of hippocampus and thalamus in schizophrenic rats

The proton magnetic resonance spectroscopy was conducted at 1 week post administration. Results showed that choline content was significantly increased in the hippocampus of schizophrenic rats compared with sham-surgery, risperidone and control groups ($P < 0.05$; Table 1, Figure 2).

Neurochemical characterization using proton magnetic resonance spectroscopy also showed that N-acetylasp-

artate and the ratio of N-acetylaspartate to creatine/phosphocreatine in the thalamus were significantly lower in the schizophrenia model rats compared with the sham-operated, risperidone and control groups ($P < 0.05$; Table 2; Figure 2). There was no significant difference in choline signals among the four groups ($P > 0.05$).

Table 1 N-acetylaspartate (NAA; machine units), choline (Cho; machine units), NAA:creatine/phosphocreatine complex (Cr), and Cho:Cr in bilateral hippocampus of *Egr3*-transfected rats

Group	NAA	Cho	NAA/Cr	Cho/Cr
Sham-operated	12.36±5.05	10.98±4.76[a]	0.83±0.67	0.80±0.58
Schizophrenia	23.60±23.26	24.12±6.79	1.12±0.91	1.32±0.79
Risperidone	24.56±6.86	12.08±3.33[a]	1.74±1.70	0.85±0.95
Control	21.93±7.76	17.40±4.67[a]	1.17±0.82	1.01±0.66

Data are expressed as mean ± SD of six rats in each group (one-way analysis of variance and Fisher's least significant difference *post-hoc* test). [a]$P < 0.05$, *vs*. schizophrenia group.

Table 2 N-acetylaspartate (NAA; machine units), choline (Cho), NAA:creatine/phosphocreatine complex (Cr), and Cho: Cr in bilateral thalamus of *Egr3*-transfected rats

Group	NAA	Cho	NAA/Cr	Cho/Cr
Sham-operated	21.46±7.29[a]	20.46±11.38	1.29±0.74[d]	1.24±1.25
Schizophrenia	10.46±4.47	20.03±18.62	0.37±0.21	0.73±0.48
Risperidone	22.75±6.50[a]	14.23±7.88	1.47±0.96[a]	1.08±0.62
Control	24.88±2.46[a]	20.40±6.29	1.68±0.58[a]	1.35±0.47

Data are expressed as mean ± SD of six rats in each group (one-way analysis of variance and Fisher's least significant difference *post-hoc* test). [a]$P < 0.05$, *vs*. schizophrenia group.

DISCUSSION

N-acetylaspartate reflects neuron integrity and activity, and a reduction in N-acetylaspartate is associated with neuronal death, defects in cell energy production, and neurite injury[28-29]. Choline signals reflect total choline content in the brain, including phosphocholine, choline glycerophosphatides, phosphatidylcholine and sphingomyelin. Various choline complexes participate in phospholipid membrane metabolism of neurons and glial cells[30-31]. Choline in tissues is elevated during cell membrane construction or degradation, possibly because of increased production of a phospholipid membrane precursor, such as phosphatidylcholine (an important component in the choline spectrum). An increase in choline may indicate a disorder of cell membrane renewal[32-33].

The creatine/phosphocreatine complex reflects the total reserve of creatine in cells. Its level is relatively stable in the brain under different metabolic conditions, so is often used as an internal standard for spectrum control. The absolute concentration of creatine/phosphocreatine complex is difficult to measure, but the ratios of N-acetylaspartate or choline to creatine/phosphocreatine are easy to obtain and relatively stable, remaining unaffected by T1 and T2 relaxation time or cerebrospinal fluid. Therefore, we measured N-acetylaspartate, choline and the ratio of N-acetylaspartate or choline to creatine/phosphocreatine to evaluate the metabolism of hippocampus and thalamus in schizophrenic rats.

Brain damage due to neural developmental disorder mainly affects the hippocampus, and some functional images indicate a significant reduction in bilateral hippocampus volume in patients with schizophrenia[21, 34-36]. However, results of studies of hippocampal N-acetylaspartate and choline content are different. Most of them suggest that the hippocampus is a characteristic site in schizophrenia. In male patients with schizophrenia, N-acetylaspartate consumption is increased in the left prefrontal lobe and thalamus. This results in a reduction in N-acetylaspartate concentration[37], found in various brain regions including the prefrontal lobe, thalamus, cingulate gyrus, and hippocampus[38-42], and may result from mitochondrial dysfunction. In addition, decreases in N-acetylaspartate and N-acetylaspartate to creatine/phosphocreatine ratio have been found in the thalamus of patients with schizophrenia[22].

Choline content was significantly greater in the schizophrenia model rats compared with the sham-operated group, indicating a disorder of cell membrane metabolism in the hippocampus of *Egr3*-transfected rats. During cell membrane construction or degradation, choline is elevated in tissues, possibly due to increased phospholipid membrane precursors, such as phosphatidylcholine[38, 43-44]. However, following risperidone treatment in *Egr3*-transfected rats, hippocampal choline content was significantly lower than in the sham-operated, risperidone and control groups. This indicates that the abnormal metabolism of hippocampal choline observed in *Egr3*-transfected rats is significantly improved by risperidone. The mechanism for these hippocampal changes is yet to be determined.

We found no statistically significant differences in the concentrations of N-acetylaspartate or choline, or the ratios of N-acetylaspartate or choline to creatine/ phosphocreatine, among the four groups. In addition, the concentration of hippocampal N-acetylaspartate was not

reduced in schizophrenia model rats.

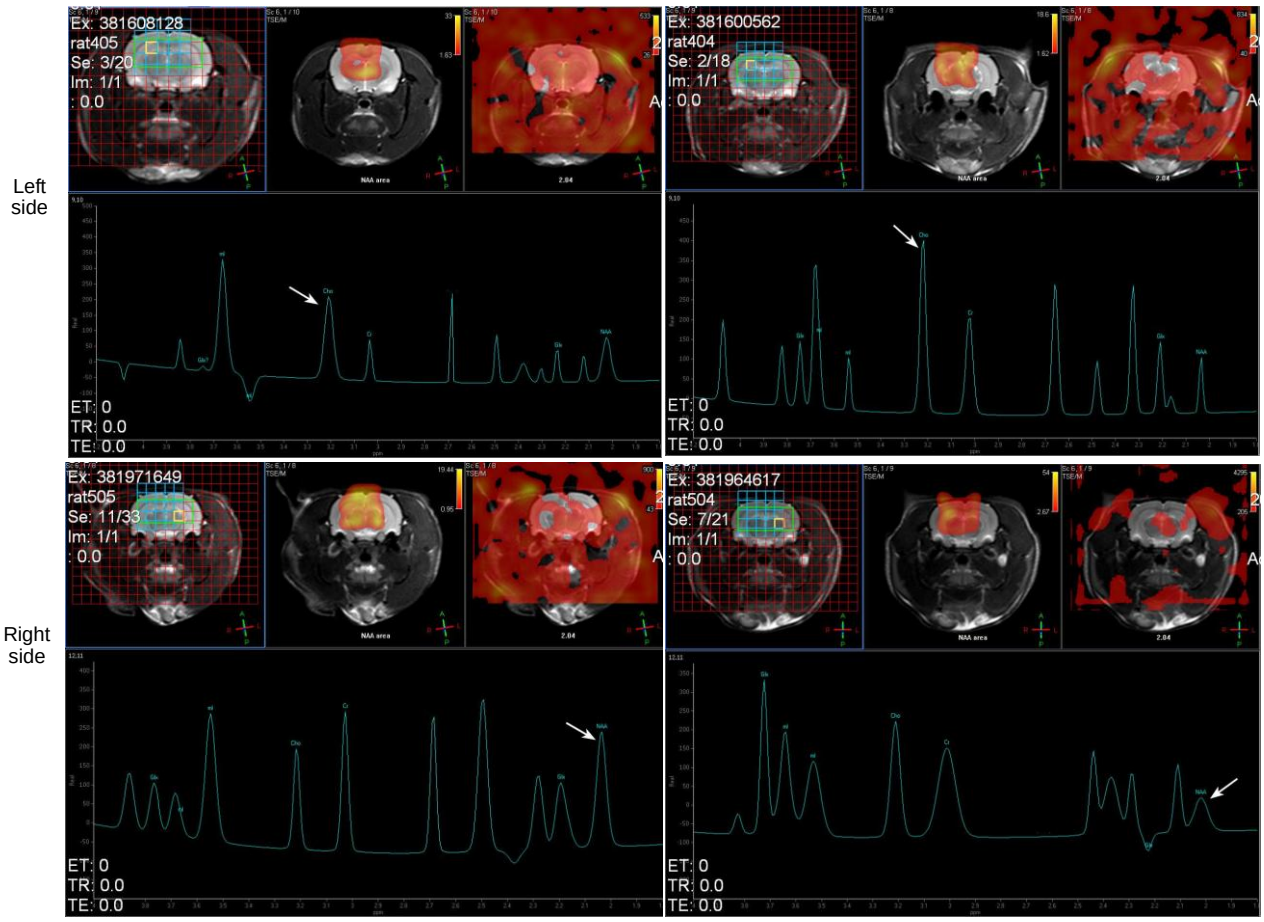

Figure 2 Proton magnetic resonance spectroscopy of the hippocampus and thalamus of *Egr3*-transfected rats.

Choline (Cho; arrow represents Cho peak) content was increased in the left hippocampus of schizophrenic rats compared with bilateral hippocampi of control group. N-acetylaspartate (NAA; arrow represents NAA peak) content was decreased in the right thalamus of *Egr3*-transfected rats compared with bilateral thalami of control group. *Egr3*: Early growth response protein 3 gene.

However, it remains unclear whether the decrease in hippocampal N-acetylaspartate concentration is associated with the severity and course of schizophrenia.

In addition, N-acetylaspartate and N-acetylaspartate to creatine/phosphocreatine ratio were significantly lower in the thalamus of *Egr3*-transfected rats than in the sham operated, risperidone, or control groups. This suggests that the integrity of thalamic neurons has been damaged, consistent with reports from studies of patients with schizophrenia. Following risperidone treatment, the thalamic concentration of N-acetylaspartate and the ratio of N-acetylaspartate to creatine/phosphocreatine complex was normalized to that of sham-operated and control groups. These results indicate that risperidone treatment significantly improved abnormal metabolism of N-acetylaspartate in schizophrenic rats. However, the precise mechanism requires further investigation.

There are some limitations to the present study. Because this study focused on schizophrenia-associated behaviors, other behavior abnormalities were not explored. In addition, we transfected bilateral hippocampi synchronously, so it is uncertain whether laterality occurs in the metabolism of N-acetylaspartate or choline in the schizophrenia model rats. In addition, we were only able to study the rats at a single time point, so it remains unclear whether the metabolic abnormalities seen in the schizophrenia model rats are related to the degree of schizophrenia-like behaviors.

In summary, we detected metabolic abnormalities in different brain regions in *Egr3*-transfected rats, a putative model of schizophrenia. Characteristics of proton magnetic resonance spectroscopy in *Egr3*-transfected rats

can provide further evidence for image-based diagnosis and for investigating the pathogenesis of schizophrenia. The metabolism of hippocampal N-acetylaspartate and thalamic choline returned to normal following risperidone treatment, indicating that risperidone is important in regulating the brain metabolism of *Egr3*-transfected rats. Proton magnetic resonance spectroscopy is a novel method of evaluating the efficacy of drugs for schizophrenia.

MATERIALS AND METHODS

Design

A randomized, controlled animal study.

Time and setting

The experiments were conducted in the animal laboratory and MRI room of Beijing Hospital, the Ministry of Public Health, China, from October 2011 to April 2012.

Materials

Animals

Twenty-four healthy, male Sprague-Dawley rats, aged 4 weeks, weighing 100 ± 10 g, were purchased from Vital River, Beijing, China (license No. SCXK (Jing) 2012-0001). Experimental protocols were conducted in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, issued by the Ministry of Science and Technology of the People's Republic of China[45].

Viral vector

Egr3 lentiviral particles were purchased from the Fuyishengke Biomedical Scientific Research Service Center, Shanghai, China. The sequence was based on NCBI Reference Sequence: NM_018781.2. The lentivirus was packaged using a four-plasmid system comprising psPAX2 (Figure 3A), pMD2G (Figure 3B), pLVX-IRES-ZsGreen1 (Figure 3C) and pLVX-IRES-ZsGreen1-*Egr-3*. pLVX-IRES-ZsGreen1 can express green fluorescent protein (Figure 3).

Methods

Egr-3 transfection

The lentivirus particle carrying the *Egr3* was injected bilaterally into the hippocampus and dentate gyrus of rats. The rats were anesthetized by intraperitoneal injection with 10% chloral hydrate (10 mL/kg), and placed in a stereotaxic frame (ST-51600, Kopf Instruments, Tujunga, CA, USA). The skin of the calvarium was sterilized with 75% alcohol, and an incision, 0.5 cm, was made. After sterilization with 0.05% hydrogen peroxide, the Bregma was exposed. Bilateral holes (0.8 mm) were drilled in the skull above the injection site using a cranial drill. The lentivirus particle was slowly injected into each side of the hippocampus (–3.0 mm anterioposterior and ± 2.0 mm mediolateral to Bregma; –2.2 mm dorsoventral to the skull surface) using a 1-μL microinjector over 20 minutes. The needles were maintained in position for further 20 minutes, and then the incision was sutured. The animals were returned to their home cages when they could move spontaneously.

Rats in the sham-surgery group (*n* = 6) underwent an identical procedure except the lentivirus particle carried green fluorescent protein instead of *Egr3*.

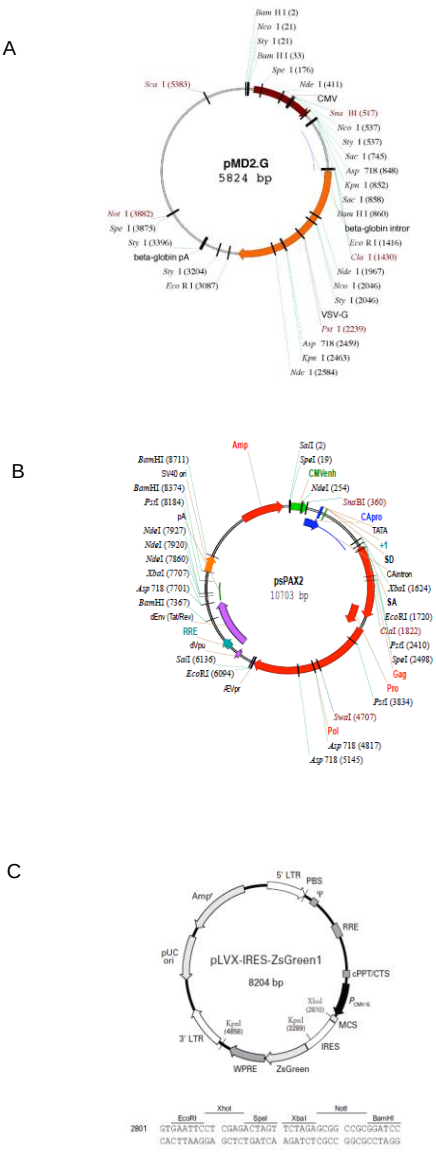

Figure 3 Plasmid graph of psPAX2 (A), pMD2G (B), and pLVX-IRES-ZsGreen1 (C) in lentivirus early growth response protein 3 particle.

After a 2-week recovery period, six of the 12 *Egr3*-transfected rats were assigned to the risperidone group and received intraperitoneal injections of risperidone (0.2 mg/kg; Sigma, St. Louis, MO, USA) for 14 consecutive days. The other three groups received intraperitoneal injections of normal saline.

In the control group (n = 6), naïve rats were injected with normal saline at each administration time point corresponding to the risperidone treatment.

Working memory of Egr3-transfected rats in Morris water maze test

The rats were evaluated in a Morris water maze (Jiliang Software Technology, Shanghai, China) working memory test to characterize the model[46-47], starting on the second day after the last injection of risperidone or saline. The water maze was a circular pool (185 cm in diameter, 45 cm in height) filled with water (23 ± 1°C), and a black platform (9 cm in diameter) was submerged to a depth of 2 cm. The water maze contained eight possible platform positions, and was divided into two groups according to the distance with the pool wall, inner (35 cm) and outer (50 cm) ring. White opaque curtains were drawn around the pool, and markers were hung on them to provide visual spatial cues to the rats. The swimming traces were monitored by an automatic tracking system (Jiliang Software Technology). Each rat received two consecutive training sessions, with a 15-second inter-session interval, for a total of 6 days. The platform position and point of entry into the water were changed at random every day. The escape latency (time taken to find the platform) was recorded. If a rat found the platform within 60 seconds, it was allowed to stay there for 15 seconds; if an animal failed to climb onto the platform within 60 seconds, it was manually guided onto the platform and allowed to remain there for 15 seconds (in this case, the escape latency was recorded as 60 seconds). The mean escape latency across all trials was calculated for each group, and the escape latency between two trials was compared to evaluate the spatial working memory of the rats.

Magnetic resonance spectrum data acquisition and analysis

A 3.0 T field strength magnetic resonance scanner (Achieva, Phillips, Netherlands) and whole-body rat coil (Shanghai Chenguang Medical Science and Technology, Shanghai, China) were used. Routine T2-weighted image sagittal, coronal, and axial scanning was conducted for all 24 rats. The maximal plane of the hippocampus and thalamus in axial and coronal planes was regarded as the central plane, and two-dimensional multi-voxel spectroscopy was conducted for the hippocampus and thalamus (Figure 4).

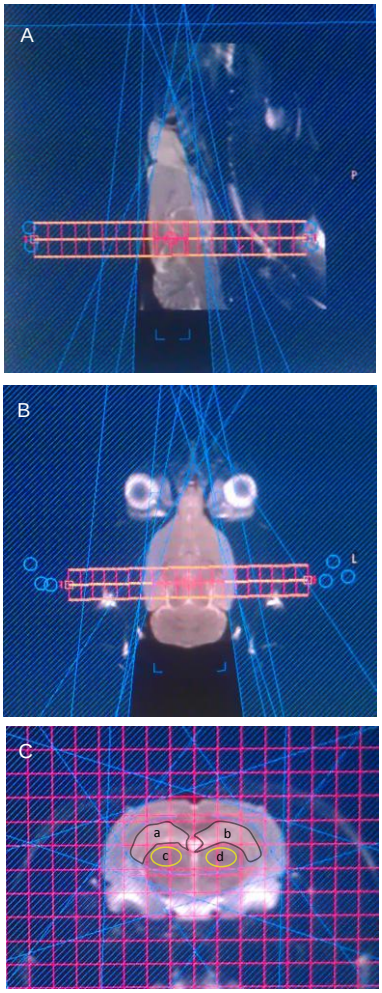

Figure 4 Hippocampal scans of a rat brain undergoing proton magnetic resonance spectroscopy.

The center of the scout image was the region of interest, surrounded by 10 saturation zones. (A) Sagittal plane; (B) axial plane; (C) coronal plane; a, b: bilateral hippocampi; c, d: bilateral thalami.

Shimming and water suppression were conducted automatically by the scanner (water suppression > 97% prior to hydrogen spectrum acquisition; repetition time, 2 000 ms; echo time, 35 ms; field of view, 40 mm × 40 mm; flip angle, 90°; slice thickness, 5 mm; number of excitations, 1; scanning time, 13 minutes and 8 seconds). Ten saturation zones were added surrounding the regions of interest after pre-tests (Figure 4), which can restrain surrounding interference and ensure the quality of images. The metabolites N-acetylaspartate, choline,

and creatine/phosphocreatine complex were detected. Spectral analysis was conducted using the built-in software package of the scanner. Baseline correction, signal average, and metabolite recognition were conducted automatically by the software, and N-acetylaspartate, choline, and ratio of choline and N-acetylaspartate to creatine/phosphocreatine (the sum of bilateral hippocampi and thalami) were calculated. The position of N-acetylaspartate was located at 2.0 ppm, choline at 3.2 ppm and creatine/phosphocreatine at 3.02 ppm. Creatine/phosphocreatine was relatively stable in one brain under different metabolic conditions, so it was regarded as a reference peak. The scanning was conducted by an investigator from the department of radiology.

Statistical analysis

Data were analyzed using SPSS 17.0 software (SPSS, Chicago, IL, USA) and expressed as mean ± SD. Mean group values were compared with one-way analysis of variance, and group differences were compared by Fisher's least significant difference *post-hoc* test. A value of $P < 0.05$ was considered statistically significant.

REFERENCES

[1] Sablier J, Stip E, Franck N. Cognitive remediation and cognitive assistive technologies in schizophrenia. Encephale. 2009,35(2):160-167.

[2] Zhang YL. Textbook of Advanced Psychiatry. Changsha, China: Central South University Press. 2007.

[3] Tsuang MT, Gilbertson MW, Faraone SV. The genetics of schizophrenia. Current knowledge and future. Directions. Schizophr Res. 1991;4(2):157-171.

[4] O'Donovan KJ, Tourtellotte WG, Millbrandt J, et al. The EGR family of transcription-regulatory factors: progress at the interface of molecular and systems neuroscience. Trends Neurosci. 1999;22(4):167-173.

[5] Eldredge LC, Gao XM, Quach DH, et al. Abnormal sympathetic nervous system development and physiological dysautonomia in Egr3-deficient mice. Development. 2008; 135(18):2949-2957.

[6] Roberts DS, Hu Y, Lund IV, et al. Brain-derived neurotrophic factor (BDNF)-induced synthesis of early growth response factor 3 (Egr3) controls the levels of type A GABA receptor alpha 4 subunits in hippocampal neurons. J Biol Chem. 2006;281(40):29431-29435.

[7] Santos AR, Duarte CB. Validation of internal control genes for expression studies: effects of the neurotrophin BDNF on hippocampal neurons. J Neurosci Res. 2008;86(16): 3684-3692.

[8] Jacobson C, Duggan D, Fischbach G. Neuregulin induces the expression of transcription factors and myosin heavy chains typical of muscle spindles in cultured human muscle. Proc Natl Acad Sci U S A. 2004;101(33):12218-12223.

[9] Calella AM, Nerlov C, Lopez RG, et al. Neurotrophin/Trk receptor signaling mediates C/EBPalpha, -beta and NeuroD recruitment to immediate-early gene promoters in neuronal cells and requires C/EBPs to induce immediate-early gene transcription. Neural Dev. 2007:2:4.

[10] Ehrengruber MU, Muhlebach SG, Sohrman S, et al. Modulation of early growth response (EGR) transcription factor-dependent gene expression by using recombinant adenovirus. Gene. 2000;258(1-2):63-69.

[11] Gao X, Daugherty RL, Tourtellotte WG. Regulation of low affinity neurotrophin receptor (p75(NTR)) by early growth response (Egr) transcriptional regulators. Mol Cell Neurosci. 2007;36(4):501-514.

[12] Dechant G, Barde YA. The neurotrophin receptor p75(NTR): novel functions and implications for diseases of the nervous system. Nat Neurosci. 2002;5(11):1131-1136.

[13] Nguyen T, Di Giovanni S. NFAT signaling in neural development and axon growth. Int J Dev Neurosci. 2008;26(2):141-145.

[14] Graef IA, Wang F, Charron F, et al. Neurotrophins and netrins require calcineurin/NFAT signaling to stimulate outgrowth of embryonic axons. Cell. 2003;113(5):657-670.

[15] Rengarajan J, Mittelstadt PR, Mages HW, et al. Sequential involvement of NFAT and Egr transcription factors in FasL regulation. Immunity. 2000;12(3):293-300.

[16] Mexal S, Frank M, Berger R, et al. Differential modulation of gene expression in the NMDA postsynaptic density of schizophrenic and control smokers. Brain Res Mol Brain Res. 2005;139(2):317-332.

[17] Yamada K, Gerber DJ, Iwayama Y, et al. Genetic analysis of the calcineurin pathway identifies members of the EGR gene family, specifically EGR3, as potential susceptibility candidates in schizophrenia. Proc Natl Acad Sci U S A. 2007;104(8):2815-2820.

[18] Gerber DJ, Hall D, Miyakawa T, et al. Evidence for association of schizophrenia with genetic variation in the 8p21.3 gene, PPP3CC, encoding the calcineurin gamma subunit. Proc Natl Acad Sci USA. 2003;100(15):8993-8998.

[19] Gallitano-Mendel A, Wozniak DF, Pehek EA, et al. Mice lacking the immediate early gene Egr3 respond to the anti-aggressive effects of clozapine yet are relatively resistant to its sedating effects. Neuropsychopharmacology. 2008;33(6):1266-1275.

[20] Cheng MC, Chuang YA, Lu CL, et al. Genetic and functional analyses of early growth response (EGR) family genes in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 2012;39(1):149-155.

[21] DeLeon A, Patel NC, Crismon ML. Aripiprazole: A comprehensive review of its pharmacology, clinical efficacy, and tolerability. Clin Ther. 2004;(26):649-666.

[22] Bustillo JR, Rowland LM, Mullins P, et al. ^{1}H-MRS at 4 Tesla in minimally treated early schizophrenia. Mol Psychiatry. 2010;15(6):629-636.

[23] Gan JL, Duan HF, Yang JM, et al. The study on correlation between brain ^{1}H-MRS findings and clinical symptoms in

patients with schizophrenia. Zhongguo Shenjing Jingshen Jibing Zazhi. 2009;35(6):359-362.

[24] Pegues MP, Rogers LJ, Amend D, et al. Anterior hippocampal volume reduction in male patients with schizophrenia. Schizophr Res. 2003;60(2-3):105-115.

[25] Szulc A, Galifiska B, Tarasow E, et al. The influence of atypical antipsychotics on brain functioning in schizophrenia. A proton magnetic resonance study. Psychiatr Pol. 2010;44(3):415-426.

[26] Wang F, Sun ZG, Ruan Y, et al. A H1- magnetic resonance spectroscopy imaging study on hippocampus in male patients with schizophrenia. Zhonghua Shenjngke Zazhi. 2004;37(2):78-80.

[27] Chen LJ, Xie SP, Chen N. A proton magnetic resonance spectroscopy on frontal lobe in the first-episode male schizophrenic patients. Shanghai Shenjing Yixue. 2006; 2(18):88-90.

[28] Hajek T, Bauer M, Pfennig A, et al. Large positive effect of lithium on prefrontal cortex N-acetylaspartate in patients with bipolar disorder: 2-centre study. J Psychiatry Neurosci. 2012;37(3):185-192.

[29] Chiu CT, Wang Z, Hunsberger JG, et al. Therapeutic potential of mood stabilizers lithium and valproic Acid: beyond bipolar disorder. Pharmacol Rev. 2013;65(1): 105-142.

[30] Lehtimäki KK, Valonen PK, Griffin JL, et al. Metabolite changes in BT4C rat gliomas undergoing ganciclovir-thymidine kinase gene therapy-induced programmed cell death as studied by 1H-NMR spectroscopy in vivo, ex vivo, and in vitro. J Biol Chem. 2003;278(46): 45915-45923.

[31] Mirbahai L, Wilson M, Shaw CS, et al. Lipid biomarkers of glioma cell growth arrest and cell death detected by 1 H magic angle spinning MRS. NMR Biomed. 2012;25(11): 1253-1262.

[32] McKnight TR, Smith KJ, Chu PW, et al. Choline metabolism, proliferation, and angiogenesis in nonenhancing grades 2 and 3 astrocytoma. J Magn Reson Imaging. 2011;33(4):808-816.

[33] Marchan R, Lesjak MS, Stewart JD. Choline-releasing glycerophosphodiesterase EDI3 links the tumor metabolome to signaling network activities. Cell Cycle. 2012:11(24):4499-506.

[34] Matsumoto H, Simmons A, Williams S, et al. Structural magnetic imaging of the hippocampus in early onset schizophrenia. Biol Psychiatry. 2001;49(10):824-831.

[35] De Peri L, Crescini A, Deste G, et al. Brain structural abnormalities at the onset of schizophrenia and bipolar disorder: a meta-analysis of controlled magnetic resonance imaging studies. Curr Pharm Des. 2012;18(4):486-494.

[36] Juuhl-Langseth M, Rimol LM, Rasmussen IA Jr, et al. Comprehensive segmentation of subcortical brain volumes in early onset schizophrenia reveals limited structural abnormalities. Psychiatry Res. 2012;30;203(1):14-23.

[37] Gan JL, Duan HF, Yang JM, et al. 1H-MRS study of prefrontal lobe and thalamus in male patients with schizophrenia. Zhongguo Yixue Yingxiang Jishu. 2009;25(7): 1152-1155.

[38] Reid MA, Stoeckel LE, White DM, et al. Assessments of function and biochemistry of the anterior cingulate cortex in schizophrenia. Biol Psychiatry. 2010;68(7):625-633.

[39] Ohrmann P, Siegmund A, Suslow T, et al. Cognitive impairment and in vivo metabolites in first-episode neuroleptic-naive and chronic medicated schizophrenic patients: a proton magnetic resonance spectroscopy study. J Psychiatr Res. 2007;41(8):625-634.

[40] Egerton A, Stone JM. The glutamate hypothesis of schizophrenia: neuroimaging and drug development. Curr Pharm Biotechnol. 2012;13(8):1500-1512.

[41] Kraguljac NV, Reid M, White D, et al. Neurometabolites in schizophrenia and bipolar disorder - a systematic review and meta-analysis. Psychiatry Res. 2012; 203(2-3):111-125.

[42] Kegeles LS, Mao X, Stanford AD, et al. Elevated prefrontal cortex gamma-aminobutyric acid and glutamate-glutamine levels in schizophrenia measured in vivo with proton magnetic resonance spectroscopy. Arch Gen Psychiatry. 2012;69(5):449-459.

[43] Francis LS, Robin AH, Katherine HT, et al. Anterior cingulate cortex: unique role in cognition and emotion. J Neuropsychiatry Clin Neurosci. 2011;23(2):121-125.

[44] Port JD, Agarwal N. MR spectroscopy in schizophrenia. J Magn Reson Imaging. 2011;34(6):1251-1261.

[45] The Ministry of Science and Technology of the People's Republic of China. Guidance Suggestions for the Care and Use of Laboratory Animals. 2006-09-30.

[46] Omata N, Chiu CT, Moya PR, et al. Lentivirally mediated GSK-3β silencing in the hippocampal DG induces antidepressant-like effects in stressed mice. Int J Neuropsychopharmacol. 2011;14(5):711-717.

[47] Zhang K, Song X, Xu Y, et al. Continuous GSK-3β overexpression in the hippocampal dentate gyrus induces prodepressant-like effects and increases sensitivity to chronic mild stress in mice. J Affect Disord. 2013;146(1): 45-52.

(Reviewed by Slone-Murphy J, Wysong S, Zhang XC, Guo G)
(Edited by Wang J, Su LL, Li CH, Song LP, Liu WJ, Zhao M)