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# Why does a high-fat diet induce preeclampsia-like symptoms in pregnant rats?

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## Research Highlights

- (1) This study proposes that a disorder of lipid metabolism may participate in the onset of preeclampsia-eclampsia during pregnancy.
- (2) Metabotropic glutamate receptor 1 exerts an effect on the occurrence of high-fat diet-induced preeclampsia during pregnancy.
- (3) The Study suggested that the control of fat intake is significant for the prevention of pregnancy-induced eclampsia.

## Abstract

Changes in neurotransmitter levels in the brain play an important role in epilepsy-like attacks after pregnancy-induced preeclampsia-eclampsia. Metabotropic glutamate receptor 1 participates in the onset of lipid metabolism disorder-induced preeclampsia. Pregnant rats were fed with a high-fat diet for 20 days. Thus, these pregnant rats experienced preeclampsia-like syndromes such as tension and proteinuria. Simultaneously, metabotropic glutamate receptor 1 mRNA and protein expressions were upregulated in the rat hippocampus. These findings indicate that increased expression of metabotropic glutamate receptor 1 promotes the occurrence of high-fat diet-induced preeclampsia in pregnant rats.

## Key Words

neural regeneration; preeclampsia; eclampsia; excitatory neurotransmitter; neurotoxicity; hyperlipidemia; hypertension; pregnancy; lipoprotein-associated phospholipase A2; metabotropic glutamate receptor 1; grants-supported paper; neuroregeneration

## INTRODUCTION

Preeclampsia is a relatively common pregnancy disorder, characterized by primary hypertension and proteinuria. In patients with severe preeclampsia, eclampsia can develop, causing nervous system symptoms

and signs<sup>[1-4]</sup>. Gestational hypertension is considered a risk factor for secondary maternal cardiovascular and cerebrovascular diseases, diabetes mellitus and filial fetal-derived adult disease<sup>[5-6]</sup>. Pregnant women with preeclampsia or eclampsia commonly have abnormal lipid metabolism combined with oxidative stress<sup>[7-11]</sup>, which can induce

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**Conflicts of interest:** None declared.

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vascular endothelial cell injury<sup>[12-14]</sup>. This injury is the basis of pathophysiological changes in pregnancy-induced preeclampsia<sup>[15-19]</sup>.

Fat deposition in pregnant women is increased to maintain the physiological needs of pregnancy, such as fetal development, labor, lactation and vernix caseosa reserves. In fact, the absorption of fat by the intestinal tract is enhanced during normal pregnancy<sup>[20-22]</sup>. Progesterone and human placental lactogen levels are increased with increased gestation<sup>[23-24]</sup>. In particular, in the third trimester of pregnancy, catabolism of adipose tissue has been shown to increase, but synthetic ability decreased, which contributed to hyperlipidemia<sup>[20-25]</sup>. Saarelainen *et al*<sup>[26]</sup> confirmed that plasma total cholesterol, triglyceride, low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol levels were higher in healthy pregnant women than those in healthy non-pregnant women. However, the ratio of low-density lipoprotein cholesterol/high-density lipoprotein cholesterol was similar between healthy pregnant women and healthy non-pregnant women, so they cannot easily suffer from angiopathy. Numerous studies<sup>[27-29]</sup> have shown that serum triglyceride, the ratio of low-density lipoprotein cholesterol/high-density lipoprotein cholesterol and free fatty acid were significantly greater in pregnant women with preeclampsia-eclampsia than those in healthy pregnant women. Abnormal increased blood lipid levels and changes in composition of lipids increase substrates for oxidative stress and enhance peroxidation. They also reduce antioxidation, increase peroxidation products and toxic substances, increase the secretion of inflammatory factors, and enhance the inflammatory reaction. These changes simultaneously combine with vascular endothelial cell injury and functional disorders, which are involved in the onset of preeclampsia and eclampsia<sup>[29]</sup>.

In the clinic, we found that some patients with preeclampsia suffered from eclampsia even with minimal blood pressure changes. Thus, the pathogenesis of hypertensive encephalopathy cannot fully explain the epilepsy-like attacks in eclampsia patients. We know that

changes in neurotransmitter levels in the brain play an important role in epilepsy-like attacks, and the imbalance between excitatory and inhibitory transmitters is a reason for epileptiform discharge<sup>[30-31]</sup>. Glutamate is the major excitatory transmitter in the central nervous system, and has been shown to be significantly increased in the cerebrospinal fluid and lesion site of epileptic patients<sup>[32-33]</sup>. The glutamate receptor is a transmembrane protein mediating the excitatory effect of glutamate. The metabotropic glutamate receptor 1 is a subtype of glutamate receptor<sup>[34]</sup>, and can promote the susceptibility to epileptic seizures<sup>[35]</sup>. Mares<sup>[36]</sup> discovered that the metabotropic glutamate receptor 1 inhibitor AIDA suppressed convulsion in filial rats. Shannon and colleagues<sup>[37]</sup> showed that the metabotropic glutamate receptor 1 selective receptor antagonist LY456236 has clinical effects in the treatment of epilepsy. Karr and Rutecki<sup>[38]</sup> suggested that administration of metabotropic glutamate receptor 1 agonist into the rat hippocampus resulted in the onset of convulsions. The above-mentioned studies indicated that metabotropic glutamate receptor 1 is strongly associated with epilepsy-like attacks. In addition, a previous study found that many epileptic patients had abnormal hippocampal structures, and epileptic seizure was strongly associated with abnormal hippocampal nerve circuits. The hippocampus is therefore a major region of interest in the study of epilepsy<sup>[39]</sup>. Therefore, the hippocampus of pregnant rats was investigated in this study. This study aimed to determine whether a high-fat diet induces abnormal lipid metabolism in pregnant rats. Furthermore, whether a high-fat diet upregulates excitatory neurotransmitter receptor expression in the hippocampus of pregnant rats or not was also investigated. Finally, the contribution that a high-fat diet makes in the development of preeclampsia symptoms in pregnant rats was investigated.

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## RESULTS

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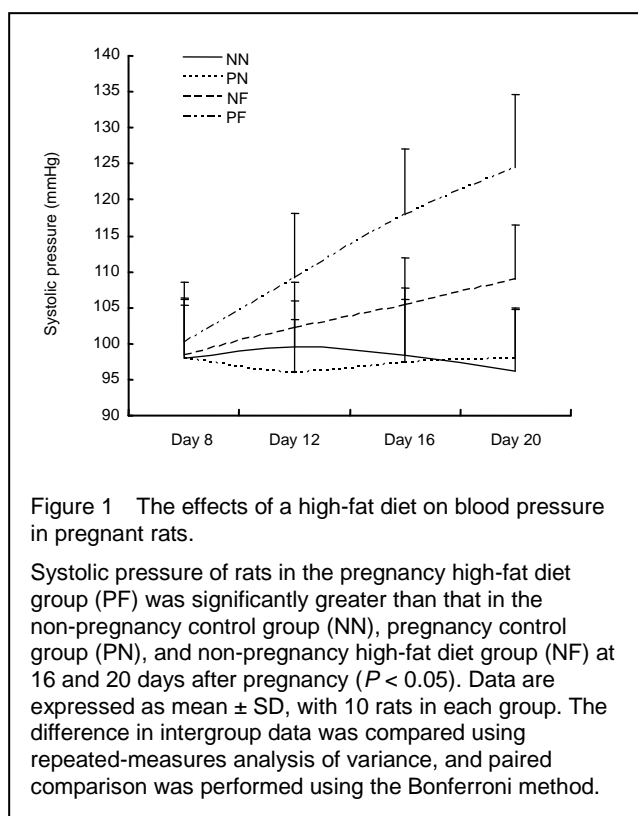
### Quantitative analysis of experimental animals

A total of 20 pregnant rats were equally and

randomly assigned to control and high-fat diet groups. Twenty non-pregnant rats were also equally and randomly as signed to control and high-fat diet groups. The rats in the control group were fed with normal feed, and those in the high-fat diet group were fed with high-fat feed. The rats in the pregnancy high-fat diet group were fed with high-fat feed immediately after conception. A total of 40 rats were included in the final analysis; no animals were excluded.

### Effects of high-fat diet on blood pressure in pregnant rats

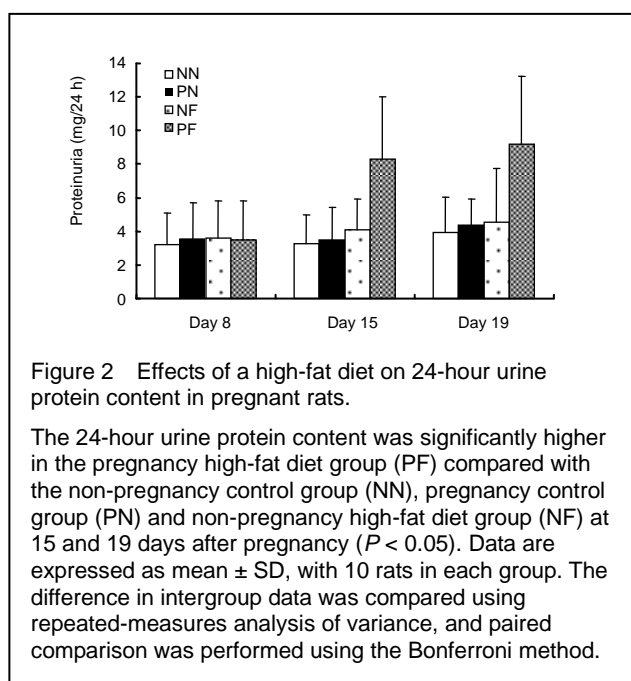
High-fat diet induced an increased blood pressure in non-pregnant and pregnant rats at 12 days after pregnancy. The blood pressure of rats in the pregnancy high-fat diet group was significantly higher than that in the non-pregnancy control group, pregnancy control group, and non-pregnancy high-fat diet group ( $P < 0.05$ ; Figure 1).



### Effects of high-fat diet on urine protein content in pregnant rats

High-fat diet induced a significant increase in 24-hour urine protein content in pregnant rats at 15 days after pregnancy. The urine protein content was significantly greater than that in the non-pregnancy control group, pregnancy control group, and non-pregnancy high-fat diet group ( $P < 0.05$ ). No significant difference in 24-hour urine protein content was detected among the

non-pregnancy control group, pregnancy control group, and non-pregnancy high-fat diet group ( $P > 0.05$ ; Figure 2).



### Effects of high-fat diet on blood lipid in pregnant rats

High-fat diet caused a significant increase in blood lipid in pregnant rats when compared with all other groups ( $P < 0.05$  or  $P < 0.01$ ; Figure 3).

### Effects of high-fat diet on metabotropic glutamate receptor 1 mRNA and protein expression in the hippocampus of pregnant rats

Reverse transcription-PCR and western blot revealed that high-fat diet significantly upregulated the expression of metabotropic glutamate receptor 1 mRNA and protein in the hippocampus of pregnant rats, with the expression being significantly higher than that in the other three groups ( $P < 0.01$ ; Figure 4).

## DISCUSSION

As previously published, this study found pregnant rats suffered from preeclampsia symptoms such as hypertension and proteinuria induced by high-fat feed<sup>[40-41]</sup>. Experimental results revealed that blood pressure and urine protein in high-fat diet pregnant rats were increased compared with normal pregnant rats, indicating that a high-fat diet could induce preeclampsia-like symptoms in rats. Levels of triglyceride, the ratio of low-density lipoprotein cholesterol/high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, free fatty acid and lipoprotein-associated phospholipase A2 were higher in

the pregnancy high-fat diet group compared with the pregnancy control group, suggesting that pregnant rats with a high-fat diet experienced a disorder of lipid metabolism, which is strongly associated with the onset of preeclampsia.

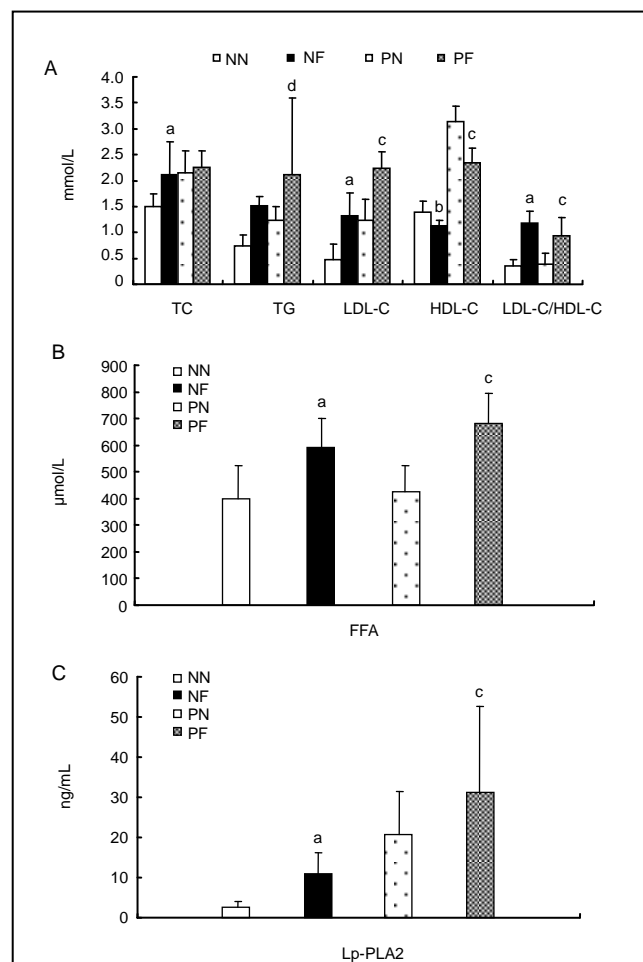


Figure 3 Effects of high-fat diet on blood lipids in pregnant rats.

(A) Serum total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) levels, and the ratio of LDL-C/HDL-C; (B) serum-free fatty acid (FFA) levels; and (C) serum lipoprotein-associated phospholipase A2 (Lp-PLA2) levels in the experimental groups.

<sup>a</sup> $P < 0.01$ , <sup>b</sup> $P < 0.05$ , vs. non-pregnancy control group (NN); <sup>c</sup> $P < 0.01$ , <sup>d</sup> $P < 0.05$ , vs. pregnancy control group (PN). Data are expressed as mean  $\pm$  SD, with 10 rats in each group. The difference in intergroup data was compared using repeated-measures analysis of variance, and paired comparison was performed using the Bonferroni method. NF: Non-pregnant high-fat diet group; PF: pregnant high-fat diet group.

Free fatty acid is a nonesterified fatty acid, released by the hydrolysis of triglycerides within adipose tissue, and can reflect an earlier disorder of blood lipid metabolism compared with triglyceride, low-density lipoprotein and high-density lipoprotein<sup>[42]</sup>. High-concentration of se-

rum-free fatty acid has been shown to reinforce oxidative stress, lead to mitochondrial dysfunction, result in excessive reactive oxygen species production, produce peroxynitrite, and injure endothelial cells<sup>[43-44]</sup>. Free fatty acid increase can activate the production of various inflammatory mediators, including tumor necrosis factor  $\alpha$  and interleukin-6, resulting in vascular endothelial injury<sup>[45-46]</sup>. These factors induced endothelial cell injury and dysfunction, promoting the development of preeclampsia. In the present study, free fatty acid content in pregnant rats with a high-fat diet was increased compared with the pregnancy control group, which was consistent with previously published studies<sup>[47-48]</sup>.

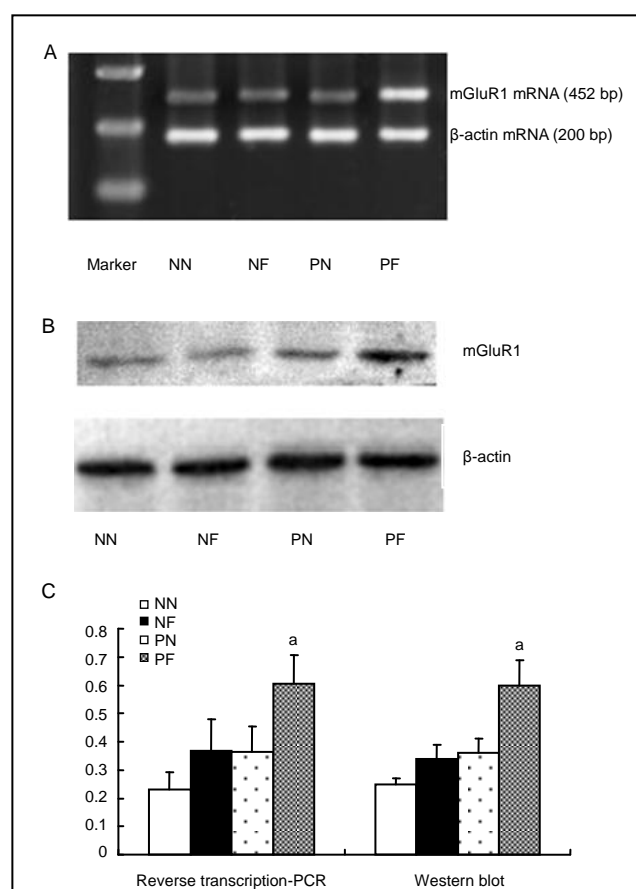


Figure 4 Effects of a high-fat diet on metabotropic glutamate receptor 1 (mGluR1) expression in the hippocampus of pregnant rats.

(A, B) Reverse transcription-PCR and western blot assay of mGluR1 expression, respectively. (C) Quantitative results of mGluR1 expression. mGluR1 expression, detected by reverse transcription-PCR and western blot assay, is presented as the gray ratio of target mRNA or target protein to  $\beta$ -actin. <sup>a</sup> $P < 0.01$ , vs. the other three groups. Data are expressed as mean  $\pm$  SD, with 10 rats in each group. The difference in intergroup data was compared using repeated-measures analysis of variance, and paired comparison was performed using the Bonferroni method. NN: Non-pregnant control group; PN: pregnant control group; NF: non-pregnant high-fat diet group; PF: pregnant high-fat diet group.

Lipoprotein-associated phospholipase A2 is an enzyme that is produced by inflammatory cells and is an inflammatory marker<sup>[49-50]</sup>. Blood lipid levels can affect the activity of lipoprotein-associated phospholipase A2<sup>[51-54]</sup>. Total cholesterol and low-density lipoprotein cholesterol obviously elevate the activity of lipoprotein-associated phospholipase A2. Triglyceride also has an activating effect. High-density lipoprotein could remarkably decrease its activity. Lipoprotein-associated phospholipase A2 is an independent factor predicting cardiovascular risk<sup>[55]</sup> and human endothelial dysfunction<sup>[56-61]</sup>. Because lipoprotein-associated phospholipase A2 is strongly associated with a disorder of lipid metabolism, and induces the inflammatory reaction, resulting in vascular endothelial cell injury, we presumed that lipoprotein-associated phospholipase A2 was possibly associated with the occurrence of preeclampsia-eclampsia. In this study, serum lipoprotein-associated phospholipase A2 was increased in the non-pregnancy high-fat diet and pregnancy high-fat diet groups compared with the non-pregnancy control and pregnancy control groups. Thus, we presumed that the binding of lipoprotein-associated phospholipase A2 to low-density lipoprotein involves apolipoprotein, produces abundant lysolecithin and oxidized free fatty acid, activates reduction-oxidation-sensitive inflammatory reaction, produces many inflammatory factors, causes vascular endothelial cell dysfunction, and participates in the occurrence of preeclampsia-eclampsia, as has been demonstrated previously<sup>[62]</sup>.

In this study, a high-fat diet induced preeclampsia-like changes in pregnant rats. Results showed hippocampal metabotropic glutamate receptor 1 expression was upregulated in the pregnancy high-fat diet group. It is presumed that increased blood lipid of pregnant rats with a high-fat diet led to vascular endothelial injury and functional disturbance, and blood-brain barrier injury. Thus, glutamate traversed the blood-brain barrier, and resulted in the occurrence of eclampsia. However, non-pregnant rats with a high-fat diet suffered from hyperlipidemia, but hippocampal metabotropic glutamate receptor 1 expression was not upregulated. Changes in endocrine levels during pregnancy may have reduced the number of protective factors for blood vessels, and aggravated vascular endothelial cell injury and blood-brain barrier injury<sup>[34]</sup>. Abundant glutamate traversed the blood-brain barrier, entered brain tissues, and affected excitatory neuroreceptors on the postsynaptic membrane, thereby producing a neurotoxic effect. This possible cascade of chemical reactions could easily cause the occurrence of eclampsia<sup>[34]</sup>. Moreover, blood lipid detection revealed that

the disorder of blood lipid metabolism was more severe in pregnant rats on a high-fat diet than that in non-pregnant rats with a high-fat diet. We believe that blood lipid levels during pregnancy plateaued at a higher level, so the range of normal blood lipid levels during pregnancy should be further quantified. This requires the addition of more samples to our future studies to identify the standard range of normal blood lipids during pregnancy, which can be used to compare body injury induced by a disorder of blood lipid metabolism between non-pregnant and pregnant stages.

In summary, a high-fat diet causes abnormal lipid metabolism during pregnancy. A disorder of lipid metabolism can cause glutamate to traverse the blood-brain barrier and induce a change in nerve excitability, all of which contributed to the occurrence of preeclampsia and eclampsia. Taken together, monitoring the changes in blood lipid during pregnancy is helpful to prevent the occurrence of preeclampsia. In future, it would be beneficial to see if a metabotropic glutamate receptor antagonist could reverse the effects of high fat diet that were just caused by the metabotropic glutamate receptor.

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## MATERIALS AND METHODS

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### Design

A randomized, controlled animal study.

### Time and setting

Experiments were performed at the Medical Experimental Animal Center, the General Hospital of Shenyang Military Region, China from May 2009 to May 2010.

### Materials

Healthy, clean, unmated male and female Wistar rats aged 8 weeks were provided by the Animal Experimental Center, the General Hospital of Shenyang Military Region, China (license No. SYXK (Jun) 2007-001). The male and female rats were housed at 18–28°C at a relative humidity of 40–70% in separate cages and were allowed free access to food and water. Subsequently, the female and male rats were randomly housed in the same cage at a ratio of 5:1. In the morning at 8:00, vaginal secretions were examined under a microscope. The finding of sperm was considered gestational day 0. After conception, the rats were housed individually. The protocols were conducted in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, formulated by the Ministry of Science and Technology of

China<sup>[63]</sup>.

## Methods

### High-fat diet

The non-pregnant high-fat diet group and pregnant high-fat diet group were given a high-fat diet (ratio: 62% fat, 18% protein and 20% carbohydrate). The non-pregnant control group and pregnant control group were given normal feed (ratio: 12% fat, 28% protein and 60% carbohydrate).

### Blood pressure measurement

Systolic pressure was measured using tail-cuff plethysmography<sup>[64]</sup>. Rats were placed at 32°C, and their blood pressure was measured using a model 59 amplifier (IITC Inc. Woodland Hills, CA, USA).

### Urine protein measurement

24-hour urine volume was collected from pregnant rats who were placed in standard metabolic cages. Urine protein levels were measured using sulfosalicylic acid turbidimetry<sup>[64]</sup>.

### Blood lipid measurement

After opening the abdominal cavity, organs were moved to the left side using forceps. After stripping the fascia surrounding the abdominal aorta, the proximal part of the abdominal aorta was pressed using the forefinger of the left hand to prevent blood spurting during puncture. A 10-mL syringe was used to take 4–5 mL blood from the crotch of the abdominal aorta with the right hand. When blood appeared, the left hand was raised and the syringe was slowly filled with the right hand. Enzyme-linked immunosorbent assay<sup>[65]</sup> was used to detect serum lipoprotein-associated phospholipase A2 and serum-free fatty acid. An auto chemistry analyzer (model 7600; Hitachi, Tokyo, Japan) was used to detect serum triglyceride, total cholesterol, low-density lipoprotein and high-density lipoprotein levels.

### Western blot assay for hippocampal metabotropic glutamate receptor 1 protein expression

At gestational day 20, five rats were obtained from each group. Under anesthesia with 10% chloral hydrate (2 mL *via* intraperitoneal injection), rats were decapitated, and brain tissue was collected. Subsequently, the hippocampus was obtained, placed in a glass homogenizer, homogenized with 10-fold volume buffer solution (137 mmol/L NaCl, 20 mmol/L Tris, 1.5 mmol/L sodium orthovanadate, 1% NP-40, 10% glycerol, 1 mmol/L phenylmethyl sulfonyl fluoride, 10 mg/L aprotinin and 0.2 mg/L leupeptin) for 10 minutes on ice, and then allowed to stand without stirring for 1 hour, followed by a

centrifugation at 14 000 r/min and 4°C for 10 minutes. The supernatant was stored at –80°C. The protein concentration was measured using the Bradford method<sup>[66]</sup>. An equal volume of 2 × sodium dodecyl sulfate buffer solution was added to the samples, which was boiled at 100°C for 5 minutes. Samples were then placed in ice water, and electrophoresed in 5% stacking gel and 7.5% separation gel (60 V stacking gel and 120 V separation gel). A total of 60 µg samples were placed in each well. After electrophoresis, the gel was placed in buffer for 30 minutes. The chamber was placed in ice water in a refrigerator, at 400 mA constant current, for 4 hours. The protein was transferred onto a nitrocellulose membrane (Pal), followed by decolorization with Tris-buffered saline containing 0.1% Tween-20. The samples were blocked with 5% skim milk and Tris-buffered saline containing 0.1% Tween-20 at room temperature for 2 hours. The membrane was subsequently incubated with rabbit anti-metabotropic glutamate receptor 1 and beta-actin polyclonal antibody (1:250 diluted; Neo-Markers Inc., Fremont, CA, USA), sealed in a plastic bag, and rolled slowly on a vertical plane at 4°C for 36 hours. After washes with Tris-buffered saline containing 0.1% Tween-20 (10 minutes × 4), horseradish peroxidase-labeled goat anti-rabbit secondary antibody (1:20 000 diluted; Proteintech Group Inc., Chicago, IL, USA) was added at room temperature for 3 hours. After washes with Tris-buffered saline containing 0.1% Tween-20 (10 minutes × 4) and Tris-buffered saline for 5 minutes, enhanced chemiluminescence reagent (Pierce, Rockford, IN, USA) was added, and colored film was scanned. Gray values of each band were measured using QuantityOne software (Bio-Rad, Hercules, CA, USA). The results were expressed as the gray ratio of target protein to β-actin.

### Reverse transcription-PCR for hippocampal metabotropic glutamate receptor 1 mRNA expression

In accordance with the TRIzol method<sup>[67]</sup>, total RNA was extracted from the hippocampus. Total RNA (1 µg) was used, and Oligo dT-Adaptor Primer served as a specific primer. Reverse transcription conditions were as follows: 42°C, 30 minutes; 99°C, 5 minutes; 5°C, 5 minutes. β-actin (10 µmol/L) was considered as an internal reference. The ratio of metabotropic glutamate receptor 1 primer to β-actin primer was 8:1. PCR of both markers was added in the same reaction system. The remaining reagents were added in accordance with the kit instructions. Primer sequences are listed in Table 1.

PCR conditions were as follows: 94°C, 2 minutes; 94°C, 30 seconds; 52°C, 30 seconds; 72°C, 2 × 5 minutes, total

30 cycles, followed by 72°C, 10 minutes. Reaction products were electrophoresed in 1.5% agarose gel. Image J software (National Institutes of Health; New York, NY, USA) was used to collect images, and then gray values were measured. The ratio of metabotropic glutamate receptor 1 to  $\beta$ -actin gray values was considered as a relative expression of metabotropic glutamate receptor 1 mRNA.

Table 1 Primer sequences of metabotropic glutamate receptor 1 and  $\beta$ -actin mRNAs

Gene	Prime (5'-3')	Product size (bp)
Metabotropic glutamate receptor 1	Forward: CAT GCC CAT TTT GTC CTA CC	452
	Reverse: CTT CTT TCT CCG GAA AAT GTT G	
$\beta$ -actin	Forward: AGA GCT ACG AGC TGC CTG AC	200
	Reverse: AGT ACT TGC GCT CAG	

### Statistical analysis

Data were analyzed using SPSS 13.0 software (SPSS, Chicago, IL, USA), and the results were expressed as mean  $\pm$  SD. The difference in intergroup data was compared using repeated-measures analysis of variance, and paired comparison was performed using the Bonferroni method. A value of  $P < 0.05$  was considered statistically significant.

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