Research report

Chronic unpredictable mild stress induces parallel reductions of 15-PGDH in the hypothalamus and lungs in rats

Jian-You Guo a, Hua Bian b, Ying Yao c, *

a Key Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences, Beijing 100101, China
b The Medical Experimental Center of Nanyang Institute of Technology, Nanyang 473004, China
c Department of Pharmacy, Zhejiang Medical College, Hangzhou 310053, China

HIGHLIGHTS

• The 15-PGDH level was decreased in the hypothalamus of stressed rats.
• The 15-PGDH level was not detected in the frontal cortex and hippocampus in controls and stressed rats.
• The 15-PGDH level was decreased in the lung of stressed rats.
• The PGE2 productions were all increased in the hypothalamus, frontal cortex, hippocampus and serum of stressed rats.

ARTICLE INFO

Article history:
Received 7 January 2015
Received in revised form 5 March 2015
Accepted 6 March 2015
Available online 14 March 2015

Keywords:
Stress
Anhedonia
Prostaglandin E2
15-PGDH
Hypothalamus

ABSTRACT

Prostaglandin E2 (PGE2) is an important inflammatory mediator and considered to be involved in the pathophysiology of depression. Previous studies that investigated the role of PGE2 in depression solely concentrated on the cyclooxygenase-dependent synthesis of this bioactive lipid. However, enzymes that degrade PGE2, such as NAD+-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH), have not yet been explored. The present study examined the expression of 15-PGDH in an animal model of depression. Depressive-like behaviors were measured after rats were exposed to chronic unpredictable mild stress (CUMS). 15 PGDH mRNA and protein expression and activity and PGE2 levels were detected in the brain and lungs of stressed animals. The stressed animals exhibited decreases in body weight gain, locomotor activity in the open field, and sucrose preference. The hypothalamus and lungs had high baseline 15-PGDH mRNA and protein expression, whereas the frontal cortex and hippocampus showed no detectable 15-PGDH mRNA or protein expression. 15 PGDH mRNA and protein expression was significantly downregulated in the hypothalamus and lungs in stressed rats compared with control rats, and the enzymatic activity of 15-PGDH was correlated with protein expression levels. PGE2 concentrations in the brain and serum increased in stressed rats. These results suggest the loss of 15-PGDH expression in depression, and 15-PGDH may be a novel potential pharmacological target for the treatment of depression.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Depression is a serious, highly prevalent disease that has a significant impact on community health worldwide and impacts approximately 15% of U.S. adults during their lifetime [1]. A deficiency in noradrenergic and serotonergic neurotransmission might
play an important role in the pathology of depression [2]. Serotonin and norepinephrine reuptake inhibitors (SNRIs) are the most commonly prescribed antidepressants. However, depression patients present various responses to standard therapy, with an expected 50% of patients who show little or no response to SNRI therapy [3]. Previous studies have shown that an inflammatory process is involved in the pathogenesis of depression [4]. Prostaglandin E2 (PGE2) is a crucial mediator of inflammation. PGE2 upregulation in serum, cerebrospinal fluid, and saliva was reported in depressed patients [5,6]. An in vitro study demonstrated that PGE2 secretion from lymphocytes increased in depressed patients compared with healthy controls [7].

PGE2 is a lipid autacoid that is derived from arachidonic acid through sequential functions of cyclooxygenase (COX), which has two major isomers: COX-1 and COX-2. COX-1 is constitutively expressed in many cell types, and COX-2 is usually considered an inducible enzyme that is upregulated following stimulation by different stressors [8]. The inhibition of COX-2, therefore, might be suggested to exert an antidepressant effect. Nonsteroidal antiinflammatory drugs (NSAIDs) inhibit COX activity and are promising for the treatment of depression [8–10]. The levels of PGE2 rely on rates of biosynthesis and degradation. However, almost all previous studies that examined the role of PGE2 in depression focused solely on the COX-dependent synthesis of this bioactive lipid. A likely pathway that elevates PGE2 levels in depression might involve a decrease in the degradation of PGE2 by the enzyme NAD*-dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH).

15-PGDH catalyzes the oxidation of the 15(S)-hydroxyl group of prostaglandins [11]. This enzyme catalyzes the reversible oxidation/reduction of prostaglandins at C-15, and 15-ketoprostaglandins exhibit a dramatic reduction of biological activity [12]. The genetic deletion of 15-PGDH can result in marked increases in tissue PGE2 levels [13]. This enzyme is generally expressed in almost all cell types. However, several tissues express the highest activity, including the lungs, kidneys, and uterus [14]. Previous reports on the expression and distribution of 15-PGDH mainly concentrated on uterine biology and parturition [15,16]. Recent studies have shown that 15-PGDH plays an important role in fever and carcinogenesis [17,18].

No study of which we are aware has examined the role of PGE2 clearance or degradation in depression. To further our understanding of prostaglandin function in depression, the present study assessed the expression of 15-PGDH in an animal model of depression. Depressive-like behaviors were measured after rats were exposed to chronic unpredictable mild stress (CUMS). 15 PGDH mRNA and protein expression and activity and PGE2 levels were detected in the brain and lungs in stressed animals. 15-PGDH expression was downregulated in the hypothalamus and lungs in stressed rats compared with control rats. Enzymatic activity was correlated with its expression levels. These data suggest the involvement of a previously unrecognized pathway in depression, in which increases in PGE2 levels derive in part from a reduction of 15-PGDH expression and activity.

2. Material and methods

2.1. Animals

Male Sprague-Dawley (200–220 g) rats were obtained from Wei Tong Li Hua Lab Animal Center (Beijing, China). All of the rats were individually housed. They were maintained on a 12 h/12 h light/dark cycle (light on 8:00 AM–8:00 PM) with food and water available ad libitum. Twenty-four rats were equally divided into a stress group and control group. The 12 rats in the stress group received 21 days of CUMS, and the 12 control rats were free of stress. The experimental procedures were approved by the ethical committee of the Institute of Psychology, Chinese Academy of Sciences.

2.2. Stress procedure

The CUMS procedure was described previously [8,19]. Briefly, the rats were exposed to different stressors daily for 21 days: Day 1 (cold immobilization for 1 h at 4 °C, forced swim for 30 min at 25 °C), Day 2 (immobilization for 1 h, crowding for 23 h), Day 3 (forced cold swim stress for 5 min at 10 °C, isolation for 23 h), Day 4 (immobilization for 1 h, vibration for 1 h), Day 5 (forced swim stress for 30 min at 25 °C, cold immobilization for 1 h at 4 °C), Day 6 (forced cold swim stress for 5 min at 10 °C, crowding for 23 h), Day 7 (vibration for 1 h, isolation for 23 h). This schedule was repeated three times for a total of 21 days. Prior to the study, certain criteria were set to exclude animals because of weight loss or the possible occurrence of wounds. The rats were acclimatized to handling for 3 min once per day for 7 consecutive days before being used in the experiment.

2.3. Body weight gain

The rats were weighed on days 1, 7, 14, and 21 of the study.

2.4. Sucrose preference test

Sucrose preference tests were performed to operationally evaluate anhedonia. The sucrose preference test consisted of first removing the food and water from each rat’s cage for 20 h. Water and 1% sucrose were then placed in the cages in preweighed glass bottles, and the animals were allowed to consume the fluids freely for 1 h. Two baseline preference tests were performed, separated by at least 5 days, and the results were averaged. A sucrose preference test was also conducted following the 21 days of CUMS. On the last day of stress, the rats were deprived of water and food for 20 h. The next day onward, the rats were given a 1 h sucrose preference test. Sucrose and water consumption (g) was measured, and sucrose preference was calculated as the following: sucrose preference (%) = sucrose consumption/sucrose consumption + water consumption × 100%.

2.5. Open field test

The open field test was conducted to evaluate exploratory and anxiety-like behavior and performed after the sucrose preference test. The open field apparatus consisted of a square arena (60 cm × 60 cm with 40 cm high walls). The entire apparatus was painted black except for 6 mm white lines that divided the floor into 16 equal size squares. The squares were subdivided into peripheral and central sectors. The central sector included the four central squares (2 cm × 2 cm), and the peripheral sector contained the squares that were adjacent to the walls. The apparatus was illuminated by a low-intensity diffuse light bulb (45 W) that was situated 45 cm above the floor of the apparatus. Each animal was placed in the central square and observed for 5 min by a video camera. The videos were recorded for further analysis. The following behaviors were scored by an observer who was blind to the drug treatment: total ambulation (overall number of peripheral and central squares crossed), central ambulation (number of central squares crossed), rearing (number of times the animal stood on its hind limbs), grooming (number of times the animal made such responses as grooming the face, licking/cleaning the body, and scratching various parts of the body), immobility (time spent immobile), Anxiety-related behavior was calculated as the following: central ambulation (%) = central ambulation/total ambulation.
The number of fecal bolii that were deposited in the open field apparatus during the exploration period was counted. After each trial, the apparatus was cleaned and wiped with a 70% ethanol solution to remove urine, feces, and odor traces left by previous animals.

2.6. Elevated plus maze

The test was performed immediately after the open field test. The rats were placed into the central area of the elevated plus maze. The elevated plus maze test was performed as previously described [20]. Briefly, the apparatus consisted of two opposing open arms (50.8 cm × 10.2 cm × 1.3 cm) and two opposing closed arms (50.8 cm × 10.2 cm × 40.6 cm) that were perpendicular to the open arms. The arms were connected by a central area (10.2 cm × 10.2 cm). Four 25-W red fluorescent lights were arranged as a cross 100 cm above the maze, providing 50 lx illumination. A video camera was suspended above the maze to record the movements of the rats. The rats were individually placed in the center of the maze facing an open arm, and the number of entries into and time spent on the open and closed arms were recorded during a 5 min observation period. Arm entries were defined as the placement of all four paws into an arm. The percentage of open-arm entries and time spent in the open arm was calculated for each animal. After each trial, the apparatus was cleaned with 70% alcohol.

2.7. Blood and tissue sample collection

After the last behavioral tests, all of the rats were decapitated between 11:00 AM and 1:00 PM. Blood samples were collected immediately. The frontal cortex, hippocampus, hypothalamus, and lungs were separated on an ice-plate and stored at −80°C until analysis.

2.8. Quantitative reverse transcription polymerase chain reaction

The mRNA expression of 15-PGDH in the frontal cortex, hippocampus, hypothalamus, and lungs was measured by quantitative reverse transcription polymerase chain reaction (RT-PCR). Total RNA was purified from these regions using the TRIzol method (Invitrogen, Carlsbad, CA, USA). cDNA was synthesized from 2 µg of total RNA using an Omniscript RT kit (Qiagen). The primer sequences were the following: 15-PGDH (forward: 5′-TGAACCTGGCGTGATCCCTG-3′; reverse: 5′-AATGGAGGCTGCCTACT-3′), GAPDH (forward: 5′-TGAACCGGAAGCTCCTGG-3′; reverse: 5′-GAGCTCAAAATTTGCTTGG-3′).

Real-time PCR was performed with a QuantiTect SYBR Green PCR Kit (Qiagen) under the following conditions: initial activation at 95°C for 15 min, denaturation at 94°C for 15 s, annealing at 55°C for 20 s, and extension at 72°C for 15 s, using a single fluorescence measurement for up to 45 cycles. A melting step was included, with temperature ramps set from 40°C to 95°C. The values were normalized to GAPDH as the endogenous control. The relative content of the 15-PGDH gene was calculated using the ΔΔCT method (2−ΔΔCT) as described previously [21].

2.9. Western blot

The frontal cortex, hippocampus, hypothalamus, and lungs were placed in chilled tubes that were treated with an enzyme inhibitor. Tissues were homogenized, and Western blot was performed as previously described [8] using primary antibodies for 15-PGDH (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and β-actin (1:10,000, Santa Cruz Biotechnology, Santa Cruz, CA, USA). A secondary antibody conjugated with horseradish peroxidase (HRP; 1:5000, Bio-Rad, Hercules, CA, USA) was used. Immunoblots were visualized on X-ray film using a chemiluminescence reaction (Pierce, Rockford, IL, USA). Image analysis was performed with optical density-calibrated images using AlphaEase Stand Alone software (Alpha Innotech).

2.10. Measurement of 15-PGDH activity

15-PGDH was measured by detecting the transfer of tritium from 15(S)-[15−3H]PGE2 to glutamate by coupling 15-PGDH with glutamate dehydrogenase as described previously [22]. Briefly, the reaction mixture contained 5 µM NH4Cl, 1 µM α-ketoglutarate, 1 µM NAD+, 1 nM 15(S)-[15−3H]PGE2 (30,000 cpm), 100 µg glutamate dehydrogenase, and crude enzyme extract in a final volume of 1 ml of 50 mM Tris–HCl, pH 7.5. The reaction was allowed to continue for 10 min at 37°C and stopped by adding 0.3 ml of 10% aqueous charcoal suspension. Radioactivity in the supernatant after centrifugation at 1000 × g for 5 min was detected by liquid scintillation counting. The calculation of the amount of oxidized PGE2 was based on the assumption that no kinetic isotope effect was involved in the oxidation of the 15(S)-hydroxyl group of 15(S)-[15−3H]PGE2 as a substrate.

2.11. Measurement of PGE2 concentration

The concentrations of PGE2 in the frontal cortex, hippocampus, hypothalamus, and serum were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen, Carlsbad, CA, USA). The frontal cortex, hippocampus, and hypothalamus were weighed and then extracted in 18 volumes of hexane:2-propanol (3:2, by volume) using a glass Tenbroek homogenizer. Prostaglandins were purified from the lipid extract using a C18 Sep-Pak cartridge (Waters). The protein content was measured using a protein assay kit, and the concentrations of PGE2 in tissue and serum were determined by ELISA.

2.12. Statistical analysis

The data are expressed as mean ± SEM. The control and stress groups were compared using Student’s t-test. Values of P < 0.05 were considered statistically significant.

3. Results

3.1. Body weight gain

Body weight gain did not differ significantly between the control and stress groups after 7 days of stress exposure (Fig. 1). However, stressed animals exhibited a reduction of body weight gain compared with controls after 14 days of stress exposure (47.14 ± 9.27 g vs. 77.76 ± 12.21 g, respectively; P < 0.05). After 21 days of CUMS,
body weight gain in the stress group was significantly less than in the control group (60.65 ± 10.45 g vs. 112.80 ± 16.14 g; P < 0.01).

3.2. Sucrose preference test

Sucrose preference was similar between the two groups before stress exposure. After 21 days of CUMS, the stressed animals exhibited a decrease in sucrose solution intake (10.72 ± 1.73 g vs. 7.05 ± 1.10 g; P < 0.05), and sucrose preference was also significantly decreased (83.94 ± 11.35% vs. 59.13 ± 8.86%; P < 0.05). No significant difference in total liquid intake was observed between the control and stress groups (P > 0.05; Fig. 2).

3.3. Open field test

Compared with the control animals, total ambulation, central ambulation, and rearing time significantly increased (P < 0.01, 0.05, and 0.05, respectively), whereas the number of grooming episodes markedly decreased in stressed rats (P < 0.01). The number of fecal boli did not differ between groups (P > 0.05; Table 1).

3.4. Elevated plus maze

The stressed rats exhibited a significant decrease in the percentage of time spent on the open arms (34.17 ± 7.92% vs. 19.23 ± 3.44%; P < 0.01) and number of entries into the open arms (32.17 ± 5.37% vs. 17.56 ± 4.44%; P < 0.01) compared with the control group. No significant difference was found in the percentage of close arm entries or time spent on the closed arm between groups (P > 0.05; Fig. 3).

3.5. 15-PGDH mRNA expression

As shown in Fig. 4, the hypothalamus and lungs had high 15-PGDH mRNA expression in the control group (0.77 ± 0.11 and 1.14 ± 0.21, respectively), whereas the prefrontal cortex and hippocampus had no detectable 15-PGDH mRNA expression in the control group. After 21 days of CUMS, 15-PGDH mRNA expression was significantly downregulated in both the hypothalamus (0.23 ± 0.06; P < 0.01) and lungs (0.79 ± 0.17; P < 0.05).

3.6. 15-PGDH protein expression

We further detected 15-PGDH protein expression in the brain and lungs (Fig. 5). 15-PGDH protein levels in the frontal cortex and hippocampus were below the limit of detection in control animals, whereas the hypothalamus and lungs had relatively high levels of 15-PGDH protein (0.28 ± 0.05 and 0.47 ± 0.09, respectively). Stressed rats exhibited a marked decrease in 15-PGDH protein levels in the hypothalamus compared with controls (29.7% of control, P < 0.01). 15-PGDH protein levels in the lungs also decreased in stressed rats compared with controls (64.8% of control, P < 0.05).
**Table 1**
Behavioral data of control and stressed animals in open field test.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total ambulation</th>
<th>Central ambulation (%)</th>
<th>Rearing</th>
<th>Grooming</th>
<th>Fecal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.2 ± 8.1</td>
<td>29.4 ± 5.6</td>
<td>25.3 ± 5.4</td>
<td>5.3 ± 1.9</td>
<td>1.57 ± 0.72</td>
</tr>
<tr>
<td>CUMS</td>
<td>20.2 ± 5.6</td>
<td>16.6 ± 3.4</td>
<td>14.9 ± 4.7</td>
<td>10.9 ± 2.7</td>
<td>2.38 ± 0.98</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM of 12 rats per group.
* Significantly different from control group value, P<0.05.
** Significantly different from control group value, P<0.01.

3.8. **PGE2 production**

PGE2 production in the hypothalamus, frontal cortex, hippocampus, and serum in the control group was 37.8 ± 7.3 pg/mg, 41.8 ± 6.5 pg/mg, 50.2 ± 7.2 pg/mg, and 21.5 ± 4.4 pg/ml, respectively. After 21 days of CUMS, PGE2 production significantly increased 2.6-fold (P<0.01), 2.5-fold (P<0.01), 4.5-fold (P<0.01), and 1.8-fold (P<0.05), respectively (Fig. 7).

4. **Discussion**

In the present study, we first explored the mRNA and protein expression and activity of 15-PGDH in the brain and lungs in an animal model of depression. We found that the mRNA and protein expression and activity of 15-PGDH were significantly downregulated in the hypothalamus and lungs in stressed rats compared with control rats. 15-PGDH expression in the frontal cortex and hippocampus was not detected in either control or stressed animals. Additionally, PGE2 production markedly increased in the frontal cortex, hippocampus, hypothalamus, and serum in stressed rats.

Neurobiological research on depression is difficult to perform in humans because of ethical limitations, and animal models are a key tool for exploring the mechanisms that underlie this disorder. Chronic unpredictable mild stress has been as a valuable model of depression for decades, in which animals are consecutively exposed to different stressors to simulate a series of life stress events [23,24]. The ethological symptoms and neurobiological abnormalities that are observed in CUMS animals are very similar to those that are present in human depression [25]. Many studies have used this model to evaluate the efficacy of various antidepressants [26,27]. In the present study, we analyzed the expression of 15-PGDH in rats that were exposed to a 21-day CUMS protocol. Open field, elevated plus maze, and sucrose preference tests were performed to assess depressive-like behavior in animals. Our results showed that CUMS significantly induced depressive-like behavior in rats. The stressed
animals exhibited decreases in body weight gain, locomotor activity in the open field, and sucrose preference. These depressive-like behaviors are in accordance with previous studies that also applied CUMS in animals [20,28] and analogous to the symptoms of major depression in humans.

Although depression has been studied for many years, its cellular and molecular mechanisms have remained largely unclear. PGE₂ plays an important role in the pathophysiology of depression, and COX inhibitors have therapeutic effects in major depression [29,30]. Almost all research conducted to date has focused on COX-2 to limit excessive PGE₂ production, but steady-state levels of PGE₂ rely on the relative rates of biosynthesis and degradation. The cytochrome P-450 enzyme carbonyl reductase is nonspecific for prostaglandins and can degrade other substances, such as drugs [31]. Broad variability is seen in the activity and tissue-specific expression of carbonyl reductase. Moreover, supraphysiologic concentrations of this enzyme are necessary to degrade prostaglandins. The enzyme prostaglandin omega hydroxylase (CYP4A4) catalyzes the conversion of PGE₂ to 20-OH PGE₂. Studies of CYP4A4 have been confined to pregnancy models and are limited mainly because of the lack of applicable reagents [14]. The enzyme 15-PGDH is currently considered to be involved in the crucial first step of PGE₂ degradation. Therefore, we examined the expression of 15-PGDH in an animal model of depression.

Recent clinical and animal studies of depression have focused on such brain regions as the frontal cortex and hippocampus because these are two key brain regions that are structurally and functionally affected by stress responses and critically involved in the regulation of mood and learning/memory function [32,33]. The hypothalamus has also been implicated in the pathogenesis of depression and as a very important brain region for PGE₂ production [34]. The mRNA and protein expression and activity of 15-PGDH were assayed in these brain regions. The hypothalamus had high basal mRNA and protein expression and activity, whereas the frontal cortex and hippocampus showed no detectable expression or activity of 15-PGDH. These results are essentially in agreement with a previous study that utilized isolated rat brains [35]. This study showed that rat cerebrum and cerebellum homogenates metabolized very little PGE₂. After 21 days of CUMS, the mRNA and protein expression and activity of 15-PGDH markedly decreased in the hypothalamus.

Notably, PGE₂ is considered to be degraded partly in the brain in adult mammals [35]. Brain PGE₂ can be transported from brain tissue and enter the blood. It is then transported to the lungs through the circulation [36]. Prostaglandins that are administered intravenously are degraded very effectively by the enzyme 15-PGDH in the lungs during even a single circulation cycle. Approximately 90–98% of blood PGE₂ is inactivated by the lungs [37]. The expression of 15-PGDH in the lungs was measured in the present study. Similar to the hypothalamus, the mRNA and protein expression and activity of 15-PGDH had high baseline levels in the lungs and significantly decreased after 21-days of CUMS.

PGE₂ is a fast-acting, short-lived mediator. It has an onset of action of 5–15 min, and its duration of action is on the order of tens of minutes [38]. The half-life of PGE₂ in blood plasma is less than 60 s. In response to psychological stress, PGE₂ is synthesized by enzymes that are induced in various cell types inside and outside the brain. Thus, the physiological activity of PGE₂ is very sensitive to changes in its catabolism [39]. In the present study, PGE₂ production was detected in the brain and serum in stressed rats. The present data showed that PGE₂ levels increased more substantially in the brain than in serum after CUMS. These results indicate that PGE₂ might be mainly induced in the brain by the CUMS procedure, and 15-PGDH in the brain appears to play a major role in depression. Moreover, prostaglandins in the central nervous system may exert their actions much longer than in the circulation under conditions of stress.

15-PGDH serves an important function that involves the degradation of PGE₂. The loss of 15-PGDH decreases PGE₂ catabolism. The present data and previous reports suggest that the regulation of PGE₂ levels in depression might involve complex mechanisms, involving both an increase in COX-2 expression and the loss of 15-PGDH. The coordinated regulation of elevated PGE₂ formation and the inhibition of PGE₂ degradation may result in increased levels of PGE₂. Several hormones and substances have been shown to affect 15-PGDH activity. Estrogen and ethanol have been reported to inhibit 15-PGDH activity [40,41], whereas progesterone and indomethacin have been shown to increase its activity [22,42]. Analyses of the effects of these substances in rat models of depression would be very interesting, and we are designing such studies for the future.

In summary, the present study examined the expression of 15-PGDH in the CUMS animal model of depression. We showed that the mRNA and protein expression of 15-PGDH was significantly reduced in the hypothalamus and lungs in stressed animals compared with controls, whereas the frontal cortex and hippocampus showed no detectable 15-PGDH mRNA or protein expression. The loss of 15-PGDH expression was correlated with a reduction of enzymatic activity. Such a loss of 15-PGDH function might decrease the metabolic inactivation of PGE₂. Altogether, our data provide evidence that a reduction of PGE₂ catabolism may promote PGE₂ production. These findings may provide a novel framework for further investigating the regulation of 15-PGDH expression and function in depression.

Conflict of interest

None.

Authors' contributions

Guo and Yao designed the study and drafted the manuscript. Guo, Bian and Yao conceived of the study. All authors read and approved the final manuscript.

Acknowledgments

This project was supported by the Key New Drugs Innovation project from Ministry of Science and Technology (2012ZX09102201-018); project from Key Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences, National Natural Foundation of China (30800301, 31170992 and 31371038); Natural Science Foundation of Zhejiang (LQ14H280001) and Project supported by Key Project of Science and Technology Department of Henan Province (132102310172). The authors would thanks Dr. Jing Liang for her general help.

References


