Bacopa monnieri (Brahmi) Prevents Cognitive Deficit by Maintaining CA2/3 VGLUT1 Density of Sub-Chronic Phencyclidine Rat Model of Schizophrenia in Normal Level

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Background: *Decreased vesicular glutamate transporter type 1 (VGLUT1) has been reported in the brains of both postmortem and animal models of schizophrenia. It indicates the deficit of glutamatergic function which is implicated in the cognitive deficit in schizophrenia. Our previous study investigated that Brahmi can recover the cognitive deficit in schizophrenia by up-regulating cerebral VGLUT1 density. However, the neuroprotective effects of Brahmi have not been studied yet.*
Objective: *To study the effects of Brahmi on the prevention of cognitive deficit and cerebral VGLUT1 density in sub-chronic phencyclidine (PCP) rat model of schizophrenia.*
Material and Method: *Rats were assigned to three groups; Group-A: Control, Group-B: PCP administration and Group-C: Brahmi + PCP. Cognitive ability was represented by the Discrimination ratio (DR) calculated from novel object recognition test. VGLUT1 optical density was measured in prefrontal cortex, striatum, cornu ammonis fields 1 (CA1) and 2/3 (CA2/3) and dentate gyrus (DG) of the hippocampus using immunohistochemistry.*
Results: *DR in PCP group was significantly decreased compared with control. This occurred alongside significantly reduced VGLUT1 in prefrontal cortex and CA2/3. Brahmi + PCP group showed a significant increase in DR score compared with PCP alone; however, it was still lower than control. This occurred alongside significant increase in VGLUT1 in CA2/3.*
Conclusion: *Cognitive deficit observed in PCP-administered rats was mediated by VGLUT1 reduction in prefrontal cortex and CA2/3. Interestingly, Brahmi could prevent this cognitive deficit by maintaining VGLUT1 density in CA2/3 in normal level.*

Keywords: *Brahmi, Schizophrenia, Animal models, Novel object recognition, VGLUT1*

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Glutamate hypofunction has been reported as one of the most popular pathological mechanism in schizophrenia. Kim et al[1] proposed it as an implicated mechanism in schizophrenia based on their finding of decreased glutamate concentrations in spinal fluid of schizophrenic patients. Additionally, several clinical studies investigated that phencyclidine (PCP), a non-competitive glutamate/N-methyl-D-aspartate (NMDA) receptor antagonist, can induce schizophrenia-like psychosis including both positive (e.g. delusion, thought disorder, hallucinations, paranoia) and negative (e.g. emotional/social withdrawal, motor retardation) symptoms[2]. Regarding to these rationales, PCP has been defined as a psychotomimetic which can produce schizophrenia-like psychosis in normal subjects and aggravate psychotic symptoms in patients with schizophrenia[3]. The administration of PCP to laboratory animals has been acceptably used and defined as the most valuable method to produce schizophrenic animal model. PCP can produce various behavioral effects resemble schizophrenia[4]. PCP at low doses can produce disinhibition and a state of euphoria, paranoia and hallucinations while high dose can produce sedation, catalepsy, general anesthesia and seizures[5,6]. Several studies reported that acute dose-dependent PCP administration can induce increases in locomotor

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activity in animals[7,8]. Additionally, PCP can disrupt sensory gating which is represented by the deficit in the prepulse inhibition (PPI) task and it was also found to be decreased in schizophrenic patients[9].

Vesicular glutamate transporters (VGLUTs) are localized only on the membrane of synaptic vesicles in glutamatergic presynaptic neurons. Exocytotic release of glutamate occurs from vesicular stores and thus depends on its transport into synaptic vesicle via VGLUTs. Vesicular glutamate transporter type 1 (VGLUT1) is a potentially valuable marker of glutamatergic terminals in brain tissue and highly selective expression in excitatory presynaptic terminals. It is predominantly expressed in cerebral cortex (except layer IV), striatum and hippocampus which are the brain regions related to schizophrenia. VGLUT1 mRNA deficits have been reported in both the hippocampal formation and in the dorsolateral prefrontal cortex in schizophrenic patients[10]. Therefore, VGLUT1 has been believed to be involved in glutamatergic hypofunction in schizophrenia.

An impairment of cognitive function is a major problem found in schizophrenic patients. It contributes to the patients' functional disability and restriction on their quality of life. It could not be attenuated by either typical or atypical antipsychotic drugs. Moreover, it persists even after positive and negative symptoms have already been treated. Although atypical antipsychotics clozapine and risperidone have been reported to reverse PCP-induced deficits in object recognition[11], they are more likely to produce major side effects such as weight gain and obesity related diseases[12]. Lately, Brahmi has been reported as neuroprotective and cognitive enhancers in Alzheimer's disease[13], but none of the studies has been done in schizophrenia. Our previous study was the first to investigate its effects in schizophrenia. We found that Brahmi can be a novel therapeutic agent for treating the cognitive deficit in schizophrenic rat models by up-regulating VGLUT1 density in CA1 and CA2/3 to normal levels[14]; however, its neuroprotective effects, which has the capability of preventing cognitive impairment, was not investigated in that study.

Brahmi or *Bacopa monnieri* is a well-known traditional Indian Ayurvedic medicinal plant. It has been reported as an herbal therapeutic that has effectively boosted memory, restored cognitive deficits and improved mental function[15]. A recent study reported that long-term, orally administration of bacosides (bacoside A and B), the active saponins of Brahmi, can prevent age-associated neurodegeneration and promote healthy brain ageing in female Wistar rats[16]. As mentioned previously, Brahmi can improve memory in the patients with Alzheimer's disease and schizophrenic rat models[13,14]. However, the neuroprotective effects of Brahmi have not been investigated in schizophrenia yet. Moreover, if it does show neuroprotective effects in schizophrenia, the mechanisms of its effects are still unknown.

The main objective of this study was to assess whether administration of Brahmi was able to prevent the cognitive impairment which was developed after sub-chronic PCP administration in rats, assessed using the novel object recognition task, and on the VGLUT1 density in the prefrontal cortex, striatum and hippocampal subfields.

Material and Method

Animals

Twenty-seven male Wistar rats weighing 200-220 g were obtained from the National Animal Center, Mahidol University, Thailand. The animals were housed one per cage and maintained at 21±2°C under a 12-hour light/dark cycle with food and water available *ad libitum* in the home cage. All animals were acclimatized for 7 days before experiment. All animal procedures were carried out in accordance with Mahidol University Code of Practice and the National Institutes of Health (USA) Guidelines for treatment of laboratory animals. The protocol for the present study was approved by the Animal Research Committee of Thammasat University, Thailand. The number of project license for animal experiment in the present study is AE 008/2552.

Drugs and drug administration

Animals were assigned to three groups (n = 9/group);

1) Control group

Animals orally received vehicle solution (distilled water) daily (08:00 h) for 14 days. They then received vehicle solution (0.9% NaCl) by intraperitonial injection twice a day (08:00 and 16:00 h) for 7 days.

2) Sub-chronic PCP group

Animals orally received vehicle solution (distilled water) daily (08:00 h) for 14 days. They then received 2 mg/kg of PCP (Sigma, USA) by intraperitonial injection twice a day (08:00 and 16:00 h) for 7 days. PCP was dissolved in 0.9% NaCl.

3) Brahmi + PCP group

Animals orally received 40 mg/kg/day of Brahmi (Planetary™ Herbals) daily (08:00 h) for 14 days. They then received 2 mg/kg of PCP (Sigma, USA) by

intraperitonial injection twice a day (08:00 and 16:00 h) for 7 days. PCP and Brahmi were dissolved in 0.9% NaCl and distilled water, respectively.

Novel object recognition test

Novel object recognition tests were performed in all groups of animals a week after drugs or vehicle administration. The test took place in a room with 360 lux lighting. The apparatus consisted of a solid black plastic box (63x63x45 cm) which was placed on the floor throughout the experiment. A video recorder (Canon) was positioned on a movable trolley above the plastic box in order to record behaviour. The objects to be discriminated were made of glass, plastic or ceramic. During the task, the bottoms of objects were fixed by the adhesive tape in order not to be displaced by the animals. Three days prior to the novel object recognition test, all rats were initially habituated to the empty box for three sessions of 3 min daily. In the novel object recognition test, each rat was placed in the box and exposed for 3 min to two identical objects placed approximately 10 cm apart in the center of the box. The rat was then returned to its home cage for an hour. The box and the objects were cleaned with 70% ethanol. Both objects in the box were replaced, one with an identical object and another with a novel object. Rats were then returned to the novel object recognition box and allowed to explore the objects for 3 min. All trials were recorded and behavioral analysis was carried out blind to treatment. Object exploring included rat sniffing, licking or touching the objects. The data were expressed as the discrimination ratio (DR) calculated from the following equation; DR = [(time exploring novel object-time exploring familiar object)/total exploration time)]. The data are expressed as mean DR value $\pm$ SEM. One-way ANOVA was performed to determine the effect of treatment on DR value, followed by Dunnett *post hoc* statistical comparison of treatment group. Independent t-test was used to compare DR value between PCP and Brahmi + PCP groups. Statistical significances were defined as $p<0.05$. All statistical analysis was performed using SPSS V13 for windows (SPSS Inc., Chicago, USA).

After novel object recognition tests were undertaken, all rats were sacrificed and whole brains were removed and preceded to immunohistochemistry.

Analysis of VGLUT1 by immunohistochemistry

After all brains were removed, they were fixed in 4% paraformaldehyde. All animal brain tissues were paraffin-embedded sections which were sectioned coronally at a thickness of 5 μm then mounted onto 3-aminopropyltriethoxysilane (APES) coated glass slides. For the sectioning, levels with respect to Bregma were determined with the use of a rat brain stereotaxic atlas. The sections for prefrontal cortex were taken between Bregma 2.7 to 2.2 mm while those for striatum were taken from Bregma 0.7 mm. Sections for hippocampus were sectioned posterior to Bregma 3.3 mm. All sections were dewaxed in xylene then rehydrated in 100%, 90% and 70% ethanol and washed for 5 min in distilled water. The sections were immersed in antigen retrieval solution (1 mM EDTA in 0.1 M Tris-HCl, pH 8.0) and heated in a microwave oven on full power (850 W) for 3x5 min. The sections were left at room temperature for 30 min to cool down before washed in Tris-HCl buffer for 2x5 min, then incubated with endogenous peroxidase blocking solution (5% H_2O_2 in absolute methanol) for 30 min. The sections were washed in Tris-HCl buffer for 2x5 min before incubation for 45 min with protein blocking solution (2% normal goat serum in Tris-HCl buffer), followed by incubation at 4°C overnight with polyclonal antibody against VGLUT1, raised in guinea pig (Chemicon International Inc, Temecula, CA) at a dilution of 1:5,000 in protein blocking solution. The sections were washed for 2x5 min in Tris-HCl buffer before incubation for 1 hour with biotinylated secondary antibody (anti-guinea pig IgG) (Vector Laboratories, Burlingame, CA) at a dilution 1:200 then processed by using avidin-biotin-peroxidase complex (VECTASTAIN® *Elite* ABC-Peroxidase Kit) (Vector Laboratories, Burlingame, CA). The sections were washed for 2x5 min. Protein immunoreactivity was visualized by using the chromogen diaminobenzidine, intensified with nickel chloride (DAB) (Vector Laboratories, Burlingame, CA). The sections were dehydrated in 70%, 90%, 100% ethanol and xylene then cover slipped with DPX mounting medium for microscopy. Negative control sections were processed as for VGLUT1 immunohistochemistry except that the primary antibody was omitted. No immunostaining could be detected under these conditions. All slides were analyzed blind to diagnosis.

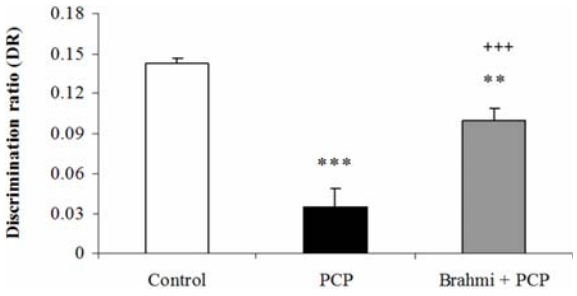
After staining for VGLUT1, the sections were scanned by Olympus microscope. VGLUT1 optical density of the regions of interest was measured using Scion Image Software based on NIH image (v. beta 3b; www.scioncorp.com; 1998). VGLUT1 optical density was made blind to the diagnostic category of the cases. Five regions of interest were measured in each of the subfields of prefrontal cortex, striatum and

hippocampus of all sections. The value measured is the sum of the optical densities of all pixels in the region divided by the number of pixels. The average of values from five regions of interest in each brain subfield of each subject was used for statistical analysis. VGLUT1 optical density of each brain region was expressed as mean optical density ± SEM. It was analyzed using one-way ANOVA with Dunnett *post hoc* comparison of treatment group. Independent t-test was used to compare VGLUT1 optical density between PCP and Brahmi + PCP groups. Statistical significances were defined as $p<0.05$. All statistical analysis was performed using SPSS V13 for windows (SPSS Inc., Chicago, USA).

Results

Novel object recognition test

One-way ANOVA with Dunnett *post hoc* tests revealed a significant reduction in the discrimination ratio in sub-chronic administration of PCP ($p<0.001$) and Brahmi + PCP ($p<0.01$) compared with control. Independent t-test revealed a significant increase in DR score in Brahmi + PCP ($p<0.001$) compared with PCP alone (Fig. 1).



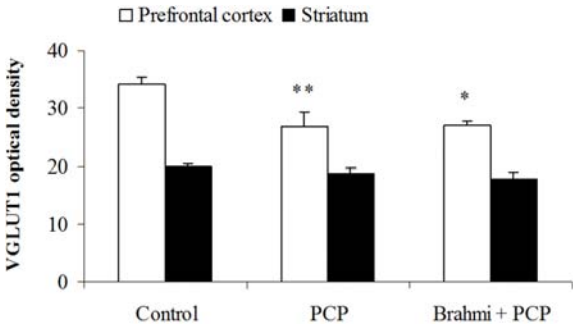
** $p<0.01$, *** $p<0.001$ vs. control, $^{+++}$ $p<0.001$ vs. PCP

Fig. 1 Discrimination ratio in control, PCP and Brahmi + PCP groups obtained from novel object recognition test. Data are mean ± SEM.

VGLUT1 immunohistochemistry

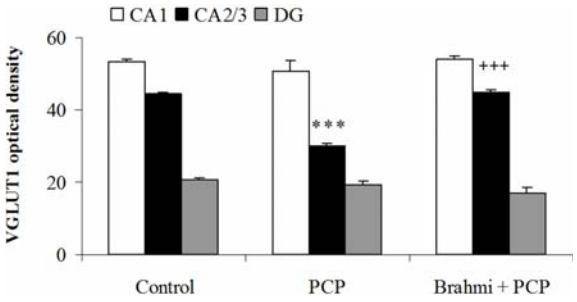
VGLUT1 is the transporter protein located on the vesicular membrane in glutamatergic presynaptic neuron in that it can be stained only in the glutamatergic terminals. Therefore, the cell bodies would be clear and not be stained for VGLUT1 in prefrontal cortex and hippocampus. Also, it is present in glutamatergic terminals in the matrix, but not in the striosomes of striatum. Immunoreactivity was not present in negative control sections in which the primary antibody was omitted.

VGLUT1 optical density was measured in the prefrontal cortex, striatum and CA1, CA2/3 and DG of the hippocampus. One-way ANOVA with Dunnett post hoc tests revealed a significant decrease in VGLUT1 optical density in prefrontal cortex in sub-chronic PCP administration group ($p<0.01$) and Brahmi + PCP group ($p<0.05$) compared with control. Independent t-test showed no significant difference between PCP alone and Brahmi + PCP groups. However, no significant difference of VGLUT1 optical density among all groups was observed in striatum (Fig. 2). Dunnett post hoc analysis also showed that VGLUT1 optical density was significantly decreased in CA2/3 of sub-chronic administration of PCP compared with control ($p<0.001$), however, no significant difference was shown between Brahmi + PCP and control. Independent t-test showed a significant increase in VGLUT1 optical density of CA2/3 in Brahmi + PCP compared with PCP alone (Fig. 3). Immunohistochemistry and bright field photo-



* $p<0.05$, ** $p<0.01$ vs. control

Fig. 2 VGLUT1 optical density from immunohistochemistry technique in prefrontal cortex and striatum in control, PCP and Brahmi + PCP groups. Data are mean optical density ± SEM.



*** $p<0.001$ vs. control, $^{+++}$ $p<0.001$ vs. PCP

Fig. 3 VGLUT1 optical density from immunohistochemistry technique in hippocampal subfields CA1, CA2/3 and DG in control, PCP and Brahmi + PCP groups. Data are mean optical density ± SEM.

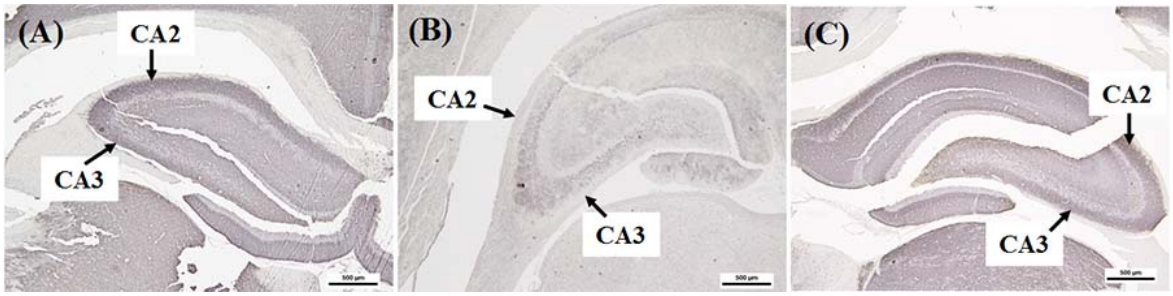


Fig. 4. Immunohistochemistry and brightfield photomicrograph of coronal sections showing VGLUT1 immunoreactivity in CA2/3 of (A) control, (B) PCP, and (C) Brahmi + PCP groups (4x magnification).

micrograph of coronal sections showing VGLUT1 immunoreactivity in CA2/3 of control, PCP and Brahmi + PCP groups was shown in Fig. 4. No significant difference of VGLUT1 optical density among all groups was observed in both CA1 and DG (Fig. 3).

Discussion

The present study insisted that sub-chronic PCP administration to animals was a valuable and well-established method that can produce the behavioral symptoms which resemble schizophrenic patients. This study revealed deficits in novel object recognition representing cognitive impairment in animals receiving sub-chronic PCP administration. The deficits in DR scores in this animal group occurred alongside the decrease in VGLUT1 optical density in prefrontal cortex and CA2/3 of the hippocampus. Our findings were consistent with the previous studies of the other researchers. They reported the decreased VGLUT1 expression in the prefrontal cortex and hippocampal formation of schizophrenic mice models and these led to impairment of working memory, social memory and sensorimotor gating[17]. Post-mortem studies also reported the reduction of VGLUT1 expression in anterior cingulate cortex[18] and prefrontal cortex[19] in schizophrenia and decreased VGLUT1 mRNA expression in entorhinal cortex of patients with major depressive disorder and bipolar disorder[20].

Normally, extracellular glutamate taken up into glial cells is metabolized to glutamine by glutamine synthetase enzyme, then transported back into neurons by glutamine transporters, converted to glutamate by glutaminase and sequestered into synaptic vesicles by VGLUTs. Glutamate is released from presynaptic terminals into the synaptic cleft by exocytosis and activates glutamate receptors on the postsynaptic membrane. To terminate the action of glutamate and maintain its extracellular concentration below excitotoxic levels, Na^+-dependent high affinity glutamate transporters (excitatory amino acid transporters, EAATs) located on the plasma membrane of neurons and glial cells rapidly remove glutamate from the extracellular space. Therefore, VGLUTs plays an important role on an amount of glutamate in presynaptic neuron and in synaptic cleft then leads to normal function of glutamatergic neurotransmission. There are three types of VGLUTs; VGLUT1, VGLUT2 and VGLUT3. VGLUT1 has been the most potentially implicated in schizophrenia because it is predominantly expressed in the cerebral cortex (except layer IV), striatum and hippocampus which are the brain regions related to schizophrenia. Thus, the present study suggested that reduced VGLUT1 in prefrontal cortex and CA2/3 indicating glutamatergic hypofunction can produce cognitive deficit in rats receiving PCP.

Glutamate has been known as an excitatory neurotransmitter which plays important roles in learning and memory. It is localized throughout the brain. Cognitive function and memory are the major obligation of prefrontal cortex and the hippocampus, respectively. Thus, the findings of the present study provided further evidence for deficits of glutamatergic innervation in prefrontal cortex and CA2/3 in schizophrenia, possibly relating to an underlying pathology of cognitive deficit in the disorder. Glutamate plays a significant role in the neuronal network of the hippocampus. The perforant path is the major input to the hippocampus. The axons of the perforant path arise principally in layers II and III of the entorhinal cortex, with minor contributions from the deeper layers IV and V. Axons from layers II/IV project to the granule cells of the DG. Thus, the findings of the present study suggested a deficit of glutamatergic innervation in the perforant path in schizophrenia. Various abnormalities in the cytoarchitecture and lamination of the hippocampus have been reported in schizophrenia[21]. Moreover, abnormal glutamate

receptor expression has been recently reported in this brain region in schizophrenia[22] which suggests an abnormality of glutamatergic neurotransmission in the hippocampus in the disorder. Therefore, the present study provided further evidence for a disturbance of subcortical glutamatergic innervation in schizophrenia.

The present study showed that Brahmi administration could prevent the cognitive deficits observed in sub-chronic PCP administration rat. Consistent with these findings, other animal studies have shown that Brahmi can prevent age-associated neurodegeneration[23]. Recent studies in humans have suggested that Brahmi extract is a potential neuroprotectant against Alzheimer's disease[24]. The prevention of cognitive deficits found in the present study occurred alongside an increased VGLUT1 density in CA2/3, but not in the prefrontal cortex.

There is evidence of antioxidant effect of Brahmi in the hippocampus which may improve capability of a task requiring the retention of new information (the recall of unrelated word pairs after a short delay). This suggests that the effect of Brahmi may be mediated by antioxidant action within the hippocampus[25]. This is consistent with the present study. Brahmi can prevent the cognitive deficit by maintaining VGLUT1 density in CA2/3 in normal level. This may be mediated by the antioxidant effect of Brahmi in the hippocampus and probably, that was why VGLUT1 cannot be maintained normally in the prefrontal cortex. However, a study of the antioxidant effect needs to be confirmed in further study.

Conclusion

While sub-chronic administration of PCP produces cognitive deficits in novel object recognition tasks and VGLUT1 reduction in prefrontal cortex and CA2/3 of the hippocampus, administration of Brahmi before receiving PCP can prevent cognitive impairment by maintaining VGLUT1 density in CA2/3 at normal levels. Therefore, Brahmi might be a novel agent that could prevent schizophrenic patients from cognitive dysfunction. However, a study of patients needs to be confirmed through further study.

What is already known on this topic?

Schizophrenia is a severe mental disorder in which there is loss of contact with reality and impaired cognitive function. It has been suggested that changes of glutamatergic neurotransmission may be involved in the pathophysiology of schizophrenia. Several studies reported the decrease of glutamate receptors and transporters (such as NMDA, EAAT3 and VGLUT1) in several brain regions in both schizophrenic post-mortem and a sub-chronic phencyclidine (PCP) rat model. Deficits in glutamate neurotransmission may lead to cognitive impairment which is a major problem contributing to the functional disability and restriction in quality of life in schizophrenic patients. Cognitive impairment cannot be attenuated by antipsychotics and can persist even after positive and negative symptoms have already been alleviated. It is thus a major problem of schizophrenic patients and it is eventually developed in all patients with schizophrenia. To date, the prevention of cognitive deficit in schizophrenia has not been investigated yet. *Bacopa monnieri* (Brahmi) is a traditional Indian Ayurvedic medicinal plant which has been used as herbal therapeutics to prevent neurodegeneration in elderly. However, the neuroprotective effects of Brahmi have not been investigated in schizophrenia yet. Moreover, if it does show the neuroprotective effects in schizophrenia, the mechanisms of its effects are still unknown.

What this study adds?

The present study is to investigate the neuroprotective effects of Brahmi on novel object recognition task in sub-chronic PCP rat model of schizophrenia. We found that Brahmi could prevent cognitive deficit as observed in schizophrenic rats. We also investigated the effects of Brahmi on VGLUT1 (Vesicular glutamate transporter type 1) density up-regulation in CA2/3 of the hippocampus in schizophrenic rats. VGLUT1 was reduced in prefrontal cortex and CA2/3 in schizophrenic rats; however, administration of Brahmi before receiving PCP can prevent cognitive deficit by maintaining the VGLUT1 density in CA2/3 at normal levels. Therefore, the present study suggested that Brahmi could be a novel neuroprotective agent for preventing cognitive deficit in schizophrenia.

Acknowledgements

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Potential conflicts of interest

None.

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Bacopa monnieri (พรมมิ) ป้องกันความบกพร่องของการเรียนรู้และความจำได้โดยทำให้ปริมาณ VGLUT1 ในสมองส่วน CA2/3 ของหนูที่ถูกเหนี่ยวนำให้เป็นโรคจิตเภทด้วย sub-chronic phencyclidine อยู่ในระดับปกติ

ปริศนา ปียะพันธุ์, ธนิศรา เวชเตง

ภูมิหลัง: *มีรายงานการลดลงของ vesicular glutamate transporter 1 (VGLUT1) ในสมองของทั้งผู้ป่วยโรคจิตเภทและหนูที่ถูกเหนี่ยวนำให้มีอาการของโรคจิตเภท การลดลงของ VGLUT1 บ่งชี้ถึงการทำงานของกลูตาเมตที่ลดลง ซึ่งมีความเกี่ยวข้องกับการบกพร่องของการเรียนรู้และความจำในผู้ป่วยโรคจิตเภท การศึกษาของเราก่อนหน้านี้ได้ พบว่าพรมมิสามารถรักษาการเรียนรู้และความจำที่ลดลงในโรคจิตเภทได้โดยการเพิ่มปริมาณ VGLUT1 ในสมอง อย่างไรก็ตามฤทธิ์ในการป้องกันความบกพร่องของการเรียนรู้และความจำของพรมมิยังไม่เคยมีการศึกษามาก่อน*

วัตถุประสงค์: *เพื่อศึกษาผลของพรมมิพืชสมุนไพรจากอินเดียต่อการป้องกันความบกพร่องของการเรียนรู้และความจำและต่อปริมาณของ VGLUT1 ในสมองของหนูที่ถูกเหนี่ยวนำให้เป็นโรคจิตเภทด้วย sub-chronic phencyclidine (PCP)*

วัสดุและวิธีการ: *หนูทดลองแบ่งเป็น 3 กลุ่ม; กลุ่ม A: กลุ่มควบคุม, กลุ่ม B: ได้รับ PCP, กลุ่ม C: พรมมิ + PCP ค่า discrimination ratio (DR) แสดงถึงความสามารถในการเรียนรู้และความจำได้มาจากการทดสอบการแยกแยะวัตถุใหม่ (novel object recognition) การวัดปริมาณของ VGLUT1 ใน prefrontal cortex, striatum, cornu ammonis fields 1 (CA1) และ 2/3 (CA2/3) และ dentate gyrus (DG) ของ hippocampus ใช้วิธี immunohistochemistry*

ผลการศึกษา: *DR ในหนูกลุ่มที่ได้รับ PCP มีค่าลดลงเมื่อเทียบกับหนูกลุ่มควบคุมการลดลงของ DR ในหนูที่ได้รับ PCP นี้เกิดขึ้นร่วมกับการลดลงของปริมาณ VGLUT1 ใน prefrontal cortex และ CA2/3 หนูกลุ่มที่ได้รับพรมมิก่อนการฉีด PCP มีค่า DR เพิ่มขึ้นอย่างมีนัยสำคัญ เมื่อเทียบกับกลุ่มที่ได้รับ PCP อย่างเดียวแต่ก็ยังมีปริมาณน้อยกว่ากลุ่มควบคุม การเพิ่มขึ้นของ DR ในหนูกลุ่มที่ได้รับพรมมิก่อนการฉีด PCP นี้เกิดขึ้นร่วมกับการเพิ่มขึ้นอย่างมีนัยสำคัญของ VGLUT1 ในสมองส่วน CA2/3*

สรุป: *การเรียนรู้และความจำที่ลดลงในหนูที่ได้รับ PCP เกิดขึ้นจากการลดปริมาณของ VGLUT1 ใน prefrontal cortex และ CA2/3 เป็นที่น่าสนใจว่าพรมมิสามารถป้องกันการลดลงของการเรียนรู้และความจำได้โดยการรักษาปริมาณของ VGLUT1 ใน CA2/3 ให้เท่ากับปกติ*
