Cognitive Enhancement Effects of *Bacopa monnieri* (Brahmi) on Novel Object Recognition and VGLUT1 Density in the Prefrontal Cortex, Striatum, and Hippocampus of Sub-Chronic Phencyclidine Rat Model of Schizophrenia

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Background: *Decreased vesicular glutamate transporter type 1 (VGLUT1) in schizophrenic brain indicates the deficit of glutamatergic function, which may produce cognitive impairment in the patients. Brahmi might be a novel therapeutic agent for the cognitive deficit treatment in schizophrenia by changing cerebral VGLUT1 density.*
Objective: *To study effects of Brahmi on attenuation at cognitive deficit and cerebral VGLUT1 density in sub-chronic phencyclidine (PCP) rat model of schizophrenia.*
Material and Method: *Rats were administered PCP or vehicle. Half of the PCP-group was treated with Brahmi. Discrimination ratio (DR) representing cognitive ability was obtained from novel object recognition test. VGLUT1 density was measured in prefrontal cortex, striatum, cornu ammonis fields 1 (CA1) and 2/3 (CA2/3) of hippocampus and dentate gyrus (DG) using western blot and immunohistochemistry.*
Results: *DR in PCP-group was significantly decreased compared with control. This occurred alongside reduced VGLUT1 in prefrontal cortex, striatum, CA1 and CA2/3. PCP with Brahmi showed a significant increase in DR score compared with PCP alone. This occurred alongside significant increase in VGLUT1 in CA1 and CA2/3.*
Conclusion: *Cognitive deficit observed in PCP-administered rats was mediated by VGLUT1 reduction in prefrontal cortex, striatum, CA1 and CA2/3. Interestingly, Brahmi could recover this cognitive deficit by increasing VGLUT1 in CA1 and CA2/3 to normal.*

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

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Glutamate hypofunction has been one of the most popular hypotheses in schizophrenia. It was first proposed as an involvement mechanism in schizophrenia by Kim et al based on their finding of reduced glutamate concentrations in spinal fluid of schizophrenic patients[1]. Additionally, clinical observations found that phencyclidine (PCP), a non-competitive glutamate/N-methyl-D-aspartate (NMDA) receptor antagonist, can induce schizophrenia-like psychosis including both positive (e.g. hallucinations, paranoia) and negative (e.g. emotional withdrawal, motor retardation) symptoms[2]. Therefore, PCP has been defined as a psychotomimetic that can produce schizophrenia-like psychosis in normal subjects and exacerbate psychotic symptoms in patients with schizophrenia[3].

PCP administration to animals has been acceptably and widely used as a valuable animal model of schizophrenia. PCP produces various behavioral effects resemble schizophrenia[4]. PCP at low dose can produce disinhibition and a state of euphoria, paranoia, and hallucinations whereas high dose can produce sedation, catalepsy, general anesthesia, and seizures[5,6]. Several studies reported the dose-dependent increases in locomotor activity in acute PCP administration to animals[7,8]. Moreover, PCP can disrupt prepulse inhibition (PPI), which is a measure of sensory gating and was found to be decreased in schizophrenia[9].

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Vesicular glutamate transporters (VGLUTs) are localized 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. VGLUT 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 involved in schizophrenia. Deficits in VGLUT1 mRNA have been reported in both the hippocampal formation and in the dorsolateral prefrontal cortex in schizophrenic patients[10]. Therefore, VGLUT1 might be involved in glutamatergic hypofunction in schizophrenia.

Cognitive deficit is a major problem found in schizophrenia. 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. Additionally, 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]. Currently, *Bacopa monnieri* or Brahmi has been reported as neuroprotective and cognitive enhancer in that it could be a novel therapeutic agent for treating the cognitive deficit in schizophrenia.

Brahmi is a traditional Indian Ayurvedic medicinal plant that has been defined as herbal therapeutics that boosts memory, restores cognitive deficits, and improve mental function[13]. Recent study has reported that long-term orally administration of bacosides, the active saponins of Brahmi, can prevent age-associated neurodegeneration and promote healthy brain ageing in female Wistar rats[14]. It has been reported that Brahmi can improve memory in the patients with Alzheimer's disease[15]. However, the cognitive enhancement effects of Brahmi have not been investigated in schizophrenia yet. Moreover, if it does show the cognitive enhancement effects in schizophrenia, the mechanisms of its effects are still unknown.

The main purpose of the present study was to assess whether administration of Brahmi was able to attenuate the effect of sub-chronic PCP administration on cognition, assessed using the novel object recognition paradigm, and on the density of VGLUT1 in the prefrontal cortex, striatum, and hippocampus.

Material and Method

Animals

Thirty-six male Wistar rats weighing 200 to 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 seven 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 = 12/group);

1. Control group

Animals received vehicle solution (0.9% NaCl) i.p. bi-daily (08:00 and 16:00 h) for seven days. They then orally received vehicle solution (distilled water) daily (08:00 h) for further 14 days.

2. Sub-chronic PCP group

Animals received 2 mg/kg of PCP i.p. bi-daily (08:00 and 16:00 h) for seven days. They then orally received vehicle solution (distilled water) daily (08:00 h) for further 14 days.

3. PCP + Brahmi group

Animals received 2 mg/kg of PCP i.p. bi-daily (08:00 and 16:00 h) for seven days. They then orally received 40 mg/kg/day of Brahmi daily (08:00 h) for further 14 days. PCP HCl (Sigma, USA) and Brahmi (Planetary™ Herbals) were dissolved in 0.9% NaCl and distilled water, respectively.

Novel object recognition test

Novel object recognition test was 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 (63 cm x 63 cm x 45 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 behavior. 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. In the three days prior to the novel object recognition test, all rats were initially habituated to the empty box for three sessions of three minutes daily. In the novel object recognition test, each rat was placed in the box and exposed for three minutes 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 three minutes. 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 ± SD. One-way ANOVA was performed to determine the effect of treatment on DR value, followed by post hoc statistical comparison of treatment group. Independent t-test was used to compare DR value between PCP and PCP with Brahmi 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 test was undertaken, all rats were sacrificed and whole brains were removed. Six of each group was preceded to western blotting experiment. The other six of each group was preceded to immunohistochemistry.

Analysis of VGLUT1 protein by western blotting technique

Prefrontal cortex, striatum, and hippocampus were prepared and stored at -80°C until the western blotting was undertaken. Tissues were homogenized in four volumes of Tris-buffered saline and centrifuged at 48,000 x g for 10 minutes at 4°C. The pellet was resuspended in four volumes of ice-cold lysis buffer. Then a protease inhibitor cocktail was added to inhibit protease activity. The homogenate was kept on ice for one hour for complete tissue lysis before centrifuged at 48,000 x g for 10 minutes at 4°C. The supernatant was collected and estimated for protein concentration by the Bradford method. Prior to electrophoresis, the supernatant was prepared with SDS-PAGE sample buffer to obtain the protein concentration of 25 µg/ml. This concentration was chosen because it would give results within the linear range for VGLUT1 estimations. The samples were boiled for five minutes and then centrifuged at 13,000 x g for three minutes and the supernatants were collected. The supernatants were analyzed on 4% stacking gel and 10% separating gel. A pooled control sample was prepared and used in one lane on each gel as an internal standard on all electrophoresis runs to control for the variation between gels. After the electrophoresis, the proteins were then electrotransferred onto polyvinylidene fluoride (PVDF) membranes (Biorad).

For VGLUT1 immunodetection, the membranes were immersed and shaken in a protein blocking solution for two hours at room temperature and incubated for one hour at 4°C with a polyclonal VGLUT1 antibody at a dilution of 1:5,000 in protein blocking solution. Then the membranes were washed with TBS buffer with 0.1% Tween 20 (TBS-Tween) for three periods of five minutes each before incubation with biotinylated secondary antibody (anti-guinea pig IgG) diluted 1:200 in protein blocking reagent at room temperature for one hour. This was followed by incubation for 30 minutes at room temperature with ABC kit (Vector) after which the membranes were washed for three periods of five minutes each with TBS-Tween. The membranes were washed for a further three periods of five minutes each with TBS alone in the final step to avoid interference with protein visualization by the detergent. The protein immunoreactivity was visualized by incubating membranes with 3, 3′, 5, 5′-tetramethylbenzidine (TMB). The scanned immunoblots were analyzed using Scion Image program (based on NIH image). The densitometry measurements were used to quantify the density of VGLUT1 band. The density obtained from the software was the integrated optical density that automatically calculated from the sum of optical densities of all pixels in the region of interest divided by number of pixels. The region of interest must completely contain the largest area of the band. The VGLUT1 immunoreactivity band was quantified by subtracting the background of membrane. The measurement was expressed as VGLUT1 optical density. VGLUT1 optical density of each brain region was analyzed using one-way ANOVA with post hoc comparison of treatment group. Independent t-test was used to compare VGLUT1 optical density between PCP and PCP with Brahmi groups.

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 five minutes 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 three periods of five minutes each. The sections were left at room temperature for 30 minutes to cool down before washed in Tris-HCl buffer for two periods of five minutes, then incubated with endogenous peroxidase blocking solution (5% H_2O_2 in absolute methanol) for 30 minutes. The sections were washed in Tris-HCl buffer for two periods of five minutes before incubation for 45 minutes 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 two periods of five minutes in Tris-HCl buffer before incubation for one 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 two periods of five minutes. 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 analyzed using one-way ANOVA with post hoc comparison of treatment group. Independent t-test was used to compare VGLUT1 optical density between PCP and PCP with Brahmi groups.

Results

Novel object recognition test

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

VGLUT1 protein by western blotting technique

One specific band of VGLUT1 protein immunoreactivity was detected at the molecular weight of 60 kDa compared with the band of pre-stained SDS-PAGE standard while no band was visualized in the negative control.

Bands of VGLUT1 immunoreactivity of all samples were measured as VGLUT1 optical density.

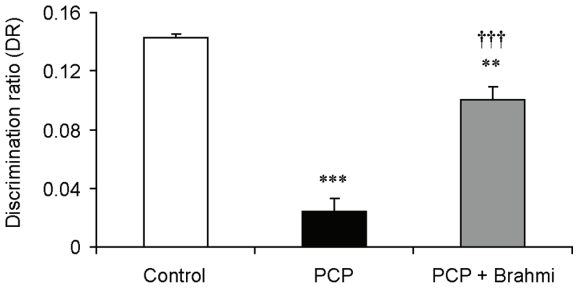

Fig. 1 Discrimination ratio in control, PCP and PCP with Brahmi groups obtained from novel object recognition test. Data are mean ± SD.
** $p<0.01$, *** $p<0.001$ vs. control, ††† $p<0.001$ vs. PCP

VGLUT1 optical density was significantly decreased in PCP compared with control in prefrontal cortex, striatum and hippocampus ($p<0.001$ in all brain areas). However, it was significantly increased in PCP with Brahmi group compared with PCP alone in all these brain areas ($p<0.001$, $p<0.05$ and $p<0.01$ in prefrontal cortex, striatum and hippocampus, respectively). VGLUT1 optical density of all brain areas was significantly decreased in PCP with Brahmi group compared with control ($p<0.05$ in prefrontal cortex and hippocampus and $p<0.01$ in striatum) (Fig. 2).

VGLUT1 immunohistochemistry

VGLUT1 protein is present in glutamatergic terminals and not in cell bodies of prefrontal cortex and hippocampus. In addition, 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 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 PCP with Brahmi group ($p<0.05$) compared with control. Independent t-test showed no significant difference between PCP alone and PCP with Brahmi groups (Fig. 3). Dunnett post hoc analysis also showed that VGLUT1 optical density was significantly decreased compared with control in sub-chronic administration of PCP in striatum ($p<0.001$), however, no significant difference was shown between PCP with Brahmi and control. No significant difference of VGLUT1 optical density was observed in PCP with Brahmi compared with PCP alone in striatum (Fig. 4). Dunnett post hoc analysis showed that VGLUT1 optical density was significantly decreased compare with control in CA1 ($p<0.001$) and CA2/3 ($p<0.001$) but not DG in sub-chronic administration of PCP. No significant difference was observed between PCP with Brahmi and control. VGLUT1 optical density of PCP with Brahmi group was significantly increased in CA1 ($p<0.01$) and CA2/3 ($p<0.001$) but not DG compared with PCP alone (Fig. 5).

Discussion

The present study showed deficits in novel object recognition in animals receiving sub-chronic PCP administration. The deficits in DR scores in

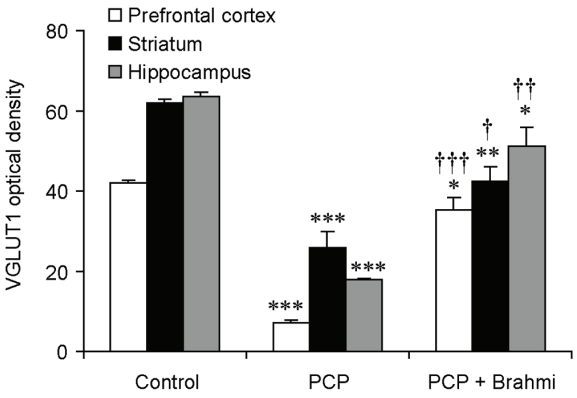

Fig. 2 VGLUT1 optical density from western blotting technique in prefrontal cortex, striatum and hippocampus of control, PCP and PCP with Brahmi groups obtained from western blotting. Data are mean ± SD.
* $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. control, † $p<0.05$, †† $p<0.01$, ††† $p<0.001$ vs. PCP

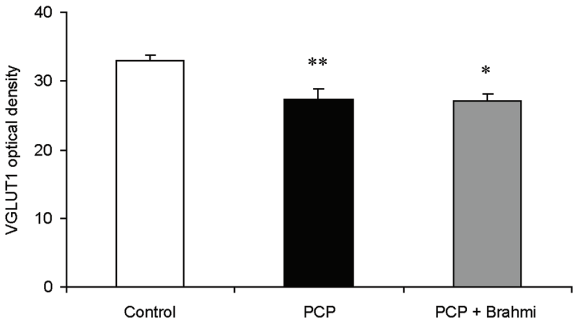

Fig. 3 VGLUT1 optical density from immunohistochemistry technique in prefrontal cortex in control, PCP and PCP with Brahmi groups. Data are mean ± SD.
* $p<0.05$, ** $p<0.01$ vs. control

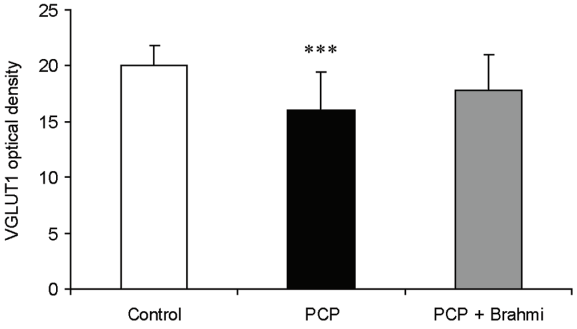

Fig. 4 VGLUT1 optical density from immunohistochemistry technique in striatum in control, PCP and PCP with Brahmi groups. Data are mean ± SD.
*** $p<0.001$ vs. control

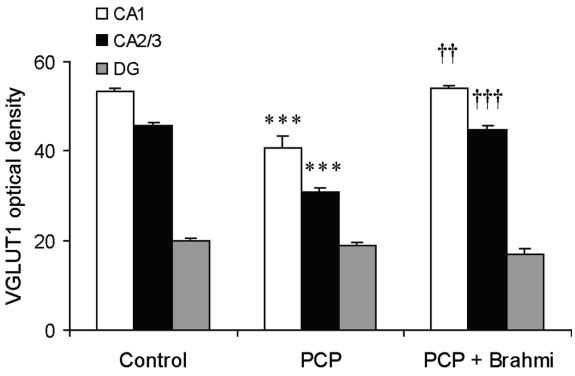

Fig. 5 VGLUT1 optical density from immunohistochemistry technique in hippocampal subfields in control, PCP and PCP with Brahmi groups. Data are mean ± SD.
*** $p<0.001$ vs. control, †† $p<0.01$, ††† $p<0.001$ vs. PCP

this animal group occurred alongside decrease in VGLUT1 optical density in prefrontal cortex, striatum, and hippocampal formation investigated by western blotting and immunohistochemistry. These findings provided additional evidence for a deficit of glutamatergic innervation in prefrontal cortex, striatum, and hippocampus in schizophrenia. These observations are consistent with studies that have reported deficits in sensorimotor gating, working and social memory in mice with reduced expression of VGLUT1 in the brain[16], as well as post-mortem studies which have reported reduced VGLUT1 expression in anterior cingulate cortex[17] and prefrontal cortex[18] in schizophrenia and decreased VGLUT1 mRNA expression in entorhinal cortex of patients with major depressive disorder and bipolar disorder[19].

Glutamate is an excitatory neurotransmitter that plays important roles in learning and memory. It is localized throughout the brain. The prefrontal regions of the cortex send glutamatergic projections to striatum[20]. Furthermore, VGLUT1 is found in the terminals of these cortico-striatal projections[21]. Thus, the findings of the present study provided further evidence for deficits of these cortico-striatal pathways in schizophrenia, possibly relating to an underlying pathology of cognitive deficit in the disorder. Glutamate also plays a significant role in neuronal network of 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 hippocampus have been reported in schizophrenia[22]. Moreover, abnormal glutamate receptor expression has been recently reported in this brain region in schizophrenia[23], which suggests an abnormality of glutamatergic neurotransmission in 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 recover the cognitive deficits observed in sub-chronic PCP administration rat. Consistent with these findings, other animal studies have shown that Brahmi could increase learning and memory task and prevent age-associated neurodegeneration[24]. Recent studies in humans have suggested that Brahmi extract is a potential cognitive enhancer and neuroprotectant against Alzheimer's disease[25]. These recovery of cognitive deficits occurred alongside an increased VGLUT1 density in CA1 and CA2/3 investigated by western blotting and immunohistochemistry. However, a significant increase of VGLUT1 optical density in prefrontal cortex and striatum after Brahmi administration compared with PCP alone was not resemble between western blotting and immunohistochemistry.

Brahmi extract has been reported as a potential cognitive enhancer and neuroprotectant[25]. Findings from the present study support a cognitive enhancement effect of Brahmi against PCP-induced VGLUT1 reduction in CA1 and CA2/3 of the schizophrenic rat model. However, effect of Brahmi administration on increased VGLUT1 density in prefrontal cortex, striatum, and DG would need to be confirmed.

Conclusion

While sub-chronic administration of PCP produces cognitive deficits in novel object recognition task and VGLUT1 reduction in prefrontal cortex, striatum, and hippocampus, administration of Brahmi provides a cognitive enhancement effect against these behavioral deficit and VGLUT1 reduction in CA1 and CA2/3 of hippocampus. Therefore, Brahmi could be a novel therapeutic agent for the cognitive deficit treatment in schizophrenic rat model. However, the investigation in the patients would need to be confirmed in the further study.

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Potential conflicts of interest

None.

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ฤทธิ์กระตุ้นการเรียนรู้และความจำของ Bacopa monnieri (พรมมิ) ต่อการแยกแยะวัตถุใหม่ และปริมาณของ VGLUT1 ใน prefrontal cortex, striatum และ hippocampus ของหนูที่ถูกเหนี่ยวนำให้เป็นโรคจิตเภทด้วย sub-chronic phencyclidine

ปริศนา ปิยะพันธุ์, ธนิศรา เวชเตง

ภูมิหลัง: *การลดลงของ vesicular glutamate transporter 1 (VGLUT1) ในสมองของผู้ป่วยโรคจิตเภทบ่งชี้ถึงการลดลงของการทำงานของกลูตาเมตซึ่งอาจทำให้ผู้ป่วยมีความบกพร่องของการเรียนรู้และความจำ พรมมิอาจเป็นสารที่ใช้ในการรักษาความบกพร่องของการเรียนรู้และความจำที่ลดลงในโรคจิตเภทได้โดยการเปลี่ยนแปลงปริมาณของ VGLUT1 ในสมอง*

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

วัสดุและวิธีการ: *หนูได้รับ PCP หรือ vehicle ครึ่งหนึ่งของหนูที่ได้รับ PCP ได้รับการป้อนพรมมิ ค่า discrimination ratio (DR) แสดงถึงความสามารถในการเรียนรู้และความจำได้มาจากการทดสอบการแยกแยะวัตถุใหม่ (novel object recognition) การวัดปริมาณของ VGLUT1 ใน prefrontal cortex, striatum, cornu ammonis fields 1 (CA1) และ 2/3 (CA2/3) ของ hippocampus และ dentate gyrus (DG) ใช้วิธี western blot และ immunohistochemistry*

ผลการศึกษา: *DR ในหนูกลุ่มที่ได้รับ PCP มีค่าลดลงเมื่อเทียบกับหนูกลุ่มควบคุม การลดลงของ DR ในหนูที่ได้รับ PCP นี้เกิดขึ้นร่วมกับการลดลงของปริมาณ VGLUT1 ใน prefrontal cortex, striatum, CA1 และ CA2/3 หนูกลุ่มที่ได้รับ PCP ร่วมกับพรมมิมีค่า DR เพิ่มขึ้นอย่างมีนัยสำคัญเมื่อเทียบกับกลุ่มที่ได้รับ PCP อย่างเดียว การเพิ่มขึ้นของ DR ในหนูกลุ่มที่ได้รับ PCP ร่วมกับพรมมินี้เกิดขึ้นร่วมกับการเพิ่มขึ้นอย่างมีนัยสำคัญของ VGLUT1 ในสมองส่วน CA1 และ CA2/3*

สรุป: *การเรียนรู้และความจำที่ลดลงในหนูที่ได้รับ PCP เกิดขึ้นจากการลดปริมาณของ VGLUT1 ใน prefrontal cortex, striatum, CA1 และ CA2/3 เป็นที่น่าสนใจว่าพรมมิสามารถฟื้นฟูการเรียนรู้และความจำที่ลดลงนี้ได้ โดยการเพิ่มปริมาณของ VGLUT1 ใน CA1 และ CA2/3 ให้กลับสู่ปกติ*