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쥐오줌풀 추출물이 MIA동물모델에서의 신경발달 단백질의 발현과 행동증상에 미치는 영향

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Effect of *Valeriana fauriei* Extract on the Neurodevelopmental Proteins Expression and Behavioral Patterns in Maternal Immune Activation Animal Model

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ABSTRACT

Background: Prenatal exposure to infectious and/or inflammatory insults can increase the risk of developing neuropsychiatric disorder such as bipolar disorder, autism, and schizophrenia later in life. We investigated whether *Valeriana fauriei* (VF) treatment alleviates prepulse inhibition (PPI) deficits and social interaction impairment induced by maternal immune activation (MIA).
Methods and Results: Pregnant mice were exposed to polyriboinosinic-polyribocytidilic acid (5 ㎎/㎏, viral infection mimic) on gestational day 9. The adolescent offspring received daily oral treatment with VF (100 ㎎/㎏) and injections of clozapine (5 ㎎/㎏) for 30 days starting on the postnatal day 35. The effects of VF extract treatment on behavioral activity impairment and protein expression were investigated using the PPI analysis, forced swim test (FST), open field test (OFT), social interaction test (SIT), and immunohistochemistry. The MIA-induced offspring showed deficits in the PPI, FST, OFT, and SIT compared to their non MIA-induced counterparts. Treatment with the VF extract significantly recovered the sensorimotor gating deficits and partially recovered the aggressive behavior observed in the SIT. The VF extract also reversed the downregulation of protein expression induced by MIA in the medial prefrontal cortex.
Conclusions: Our results provide initial evidence of the fact that the VF extract could reverse MIA-induced behavioral impairment and prevent neurodevelopmental disorders such as schizophrenia.

***Key Words*:** *Valeriana fauriei* Briq., Maternal Immune Activation, Polyriboinosinic-Polyribocytidilic Acid, Schizophrenia

INTRODUCTION

Valeriana fauriei Briq. (VF) has been used in humans for hundreds of years (Liu *et al*., 2012). This genus with over 250 species contains a variety of compounds, including valepotriates, valerenic acid, and its derivatives

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(Ahn *et al.*, 2012; Liu *et al*., 2012). *Valeriana* has been used for people many years in China and Korea (Lee *et al*., 2016b).

Valeriana genus has been used for centuries for treating epilepsy, myalgia, sleep disorders, and anxiety (Bent *et al*., 2006). Many studies have shown its neuroprotective effect in *in vitro* models of Parkinson's disease. An aqueous extract of *Valeriana* has significant cytoprotective effect on rotenone-induced apoptosis in human neuroblastoma SH-SY5Y cells (de Oliveria *et al*., 2009). Its anti-inflammatory properties as an inhibitor of NF-κB has emerged from the knowledge of its traditional use as an anti-inflammatory remedy in Europe (Jacobo-Herrera *et al*., 2006).

Miyasaka *et al*. (2006) have reported the efficacy and safety of valerian as a treatment option for anxiety disorders. In previous studies, the effects of VF on prenatal stress (PNS) offspring-related psychiatric disorders such as depression and schizophrenia were determined (Kang *et al*., 2014; Lee *et al*., 2016a). In addition, changes in protein levels and behavioral patterns were examined in the prefrontal cortex of PNS rats. We have revealed that the changes due to PNS are affected by VF treatment (Lee *et al*., 2016a). Considering these data and the already known effects of VF on various diseases, we proposed that VF might be a target in the search for new agents to assist treatment for psychiatric disorders.

Therefore, the objective of this study was to determine the effect of VF administration on behavioral symptoms and the expression of neurodevelopmental proteins in a MIA model.

Maternal immune activation (MIA) due to infection during pregnancy has been repeatedly implicated in the etiology of developmental neuropsychiatric disorders, including bipolar disorder (Canetta *et al*., 2014), autism (Brown *et al*., 2014), and schizophrenia (Fatemi *et al*., 2008). MIA can be induced by injecting pregnant dams with viral mimic polyriboinosinic‐polyribocytidilic acid (Poly I:C), leading to a wide spectrum of schizophrenia-relevant functional and neuropathological deficits in adult offspring (Meyer *et al*., 2010b).

Many MIA-induced behavioral, cognitive, and pharmacological dysfunctions in adult offspring are directly implicated in schizophrenia and other psychosis-related disorders, including abnormalities in sensorimotor gating, selective attention, deficits in social interaction, working memory, and sensitivity to psychostimulant drugs (Bitanihirwe *et al*., 2010).

In schizophrenia-like animal model induced by MIA, chronic antipsychotic drug treatment during periadolescence may prevent subsequent emergence of psychosis-related behavioral and pharmacological abnormalities in adulthood (Meyer *et al*., 2010b). In addition, clozapine can effectively block phencyclidine-induced hyperlocomotion, improving the disruption of prepulse inhibition and deficits in social interaction (Bakshi *et al*., 1994).

MATERIALS AND METHODS

1. Preparation of *Valeriana fauriei* extracts

The VF was obtained from a local farm in Jingbu province Republic of Korea. The roots of VF were air-dried avoiding sun-light and cut into small pieces for the experiment. The dried roots (2 ㎏) were soaked in 70% ethanol (3 ℓ) at room temperature for 1 day and extracted for 1 hour three times with 70% EtOH in an ultrasonic apparatus and filtered with filter paper (Advantec Toyo Kaisha Ltd., Tokyo, Japan) to remove the debris. The EtOH extract was evaporated under reduced pressure by rotary evaporator and lyophilized with freezing dryer to give 70% EtOH crude extract (330.2 g, yield 16.5%).

2. Animals

C57BL6/J mice (8 weeks old) were purchased from Central Lab Animal Inc. (Seoul, Korea). Mice were mated in groups of 1 male and 2 females. When a vaginal plug was observed during daily control, female mice were considered pregnant and separated. All mice were housed under standard conditions of a 12/12-h light/dark cycle (lights on at 06 : 30) with free access to food and water. All animal procedures were performed in accordance with the guidelines for the care and use of laboratory animals provided by the US National Institutes of Health (NRC, 1996).

3. Maternal immune activation and drug administration to mice

Clozapine (5 ㎎/㎏/day, Sigma-Aldrich Co., St. Louis, MO, USA) was dissolved in acetic acid and diluted with saline followed by intraperitoneal injection. VF (100 ㎎/㎏/

day) was dissolved in water and orally administered on postnatal day 35 for 4 weeks until postnatal day 65 (Meyer *et al*., 2010b).

Pregnant dams on GD 9 received either a single injection of Poly I:C or CON (saline) solution via intravenous route at the tail vein under mild physical constraint. Poly I:C (potassium salt) was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA) and dissolved in isotonic 0.9% NaCl solution to obtain the desired dosage (5 ㎎/㎏ calculated based on pure form of Poly I:C. All animals were returned to their home cages immediately after the injection procedure and left undisturbed until weaning of the offspring.

4. Behavioral tests

Modified behavioral tests, including forced-swim test (FST), open-field test (OFT), social interaction test (SIT), and prepulse inhibition (PPI) test, were performed as described previously (Lee *et al*., 2016a).

1) Prepulse inhibition

An automated startle reflex system (SR Lab, San Diego Instruments, San Diego, CA, USA) was used to measure prepulse inhibition. This system consisted of a startle chamber housed in a sound attenuated isolation cabinet equipped with an internal fan and light. A cylindrical transparent acrylic holding apparatus resting on a four-pegged platform within the isolation chamber was used to hold each subject throughout the testing session.

Background noise and acoustic stimuli were controlled via the SR Lab microcomputer and interface assembly. They were delivered through a speaker mounted above the cylindrical holding apparatus. All test chambers were located in a sound attenuated experimental room to minimize external noise (Chang *et al*., 2015). Background noise of 68 dB was present throughout the test session. After a 5 min acclimation period to the background noise, trials were presented in pseudorandom order, including 14 pulse alone trials in which a 40 ms, 120 dB broadband noise burst was presented, 30 prepulse + pulse trials in which the onset of a 20 ms broadband noise prepulse preceded the onset of the 120 dB pulse by 100 ms (10 for each of prepulse intensities of 3, 6, and 12 dB above the background noise, respectively), and eight non-stimulus trials consisting of only background noise. Prepulse intensities used in our protocol did not induce startle reaction. All trials were presented with an average inter-trial interval of 22 s (15 - 30 s range). Four 120 dB pulse trials were presented at the beginning and the end of the test session with a series of 60 acoustic stimuli trials. However, they were not used in the calculation of PPI values.

The holding chambers were cleaned with 75% ethanol between each test session. The level of PPI was calculated as a percentage score for each prepulse using the following formula: %PPI = 100 - [{(startle response for prepulse + pulse trial) / (startle response for pulse alone trial)} × 100] (Nozari *et al*., 2015). Mean %PPI was considered as an overall measure of the observed treatment for which percent PPI data were averaged for three prepulses (Meyer *et al*., 2010b).

2) Forced-swim test (FST)

FST was conducted as described previously (Adachi *et al*., 2008). Mice were gently placed in a large transparent cylinder filled with fresh warm tap water (25 ± 2℃) for 5 min. The water was changed between mice. The behaviors of swimming, climbing, and immobility were recorded with a video camera by an observer with a stopwatch.

3) Open-field test (OFT)

OFT was used to assess exploratory activity and reactivity to a novel environment. The test was carried out in clear opaque Plexiglas boxes (50 ㎝ × 50 ㎝ × 25 ㎝) equipped with a video as described previously (Cryan and Mombereau, 2004). Mice were placed in the center of the apparatus and their locomotor behaviors were recorded for 20 min. Horizontal locomotor activity was expressed as total ambulatory distance. The test box was cleaned with 70% ethanol between tests.

4) Social interaction test (SIT)

SIT was adapted from previous studies (Zhu *et al*., 2014). Using the same open-field box, before the experiment, mice were placed in the environment for 30 min for acclimatization. SIT is commonly referred to as the behavior that occurs in a social context resulting from interaction between and among individuals (of the same species with equal body weight).

The behaviors of mice were recorded by a video camera placed above the arena. Social interaction included

the following behaviors; following or approaching the test partner, mounting or biting the test partner, sniffing or grooming any part of the body of the test partner. Each session lasted 20 min. Total duration of social play and the numbers and types of interactions were recorded.

5. Western blot

Medial prefrontal cortex (mPFC) tissues were lysed in RIPA buffer containing protease inhibitors followed by centrifugation at 14,000 rpm for 10 min at 4℃.

To detect dihydropyrimidinase-like 2 (Dpysl2) and neurofilament protein, 40 g of lysed protein was subjected to 10 and 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane (Merck Millipore, Billerica, MA, USA). After blocking with 5% skim milk, membranes were probed with anti-Dpysl2 (1 : 1,000, Cell Signaling Technology Inc., Danvers, MA, USA), anti-LIM and SH protein 1 (Lasp1, 1 : 2,000, Merck Millipore, Billerica, MA, USA), anti-neurofilament M (Nefm, 1 : 1,000, Cell Signaling Technology Inc., Danvers, MA, USA), or anti-β-tubulin (Tubb, 1 : 3,000, Thermo Fisher Scientific Inc., Waltham, MA, USA) antibodies overnight at 4℃. After washing three times, membranes were then incubated with peroxidase-conjugated secondary anti-mouse (1 : 10,000, Sigma-Aldrich Co., St. Louis, MO, USA) or anti-rabbit (1 : 5,000, Abfrontier, Young In Frontier Co., Ltd., Seoul, Korea) for 1 h at room temperature. Immunoreactive bands were detected using an enhanced chemiluminescence kit (ELPIS-Biotech Inc., Daejeon, Korea). Quantitative measurements of Dpysl2, Lasp1, Nefm and Actb proteins were obtained using ImageJ software.

6. Immunohistochemistry

Mice were deeply anesthetized with ethyl ether and perfused with 4% paraformaldehyde. Fixed brains were removed, frozen, and cut into 30 ㎛ sections. Frozen sections from mPFC were blocked with normal horse serum, incubated with anti-Dpysl2 (1 : 700, Atlas Antibodies AB, Stockholm, Sweden), Nefm (1 : 100, Cell Signaling Technology, Danvers, MA, USA), and anti-NeuN (1 : 100, Merck Millipore, Billerica, MA, USA) followed by incubation with Cy3-conjugated anti-rabbit and mouse secondary antibodies (1 : 500 and 1 : 800, Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA). Fluorescent images were captured using a confocal laser-scanning microscope (FV10-ASW, Olympus Co., Tokyo, Japan). Images were quantified with Image J software using a protocol described previously with slight modifications (Kim *et al*., 2015).

7. Statistical analysis

All data are expressed as mean ± standard deviation and/or standard error of the mean. They were compared using Student's *t*-test. All statistical analyses were performed using IBM SPSS statistics 22 software (SPSS Inc, Chicago, IL, USA). p-values < 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

We used mouse MIA model to evaluate the extent to which the VF treatment altered the behavioral and protein expression that might be related to the pathophysiology of MIA-induced psychiatric disorders due to Poly I:C during pregnancy. We investigated the effects of VF treatment on MIA-induced behavioral phenotypes with PPI, FST, OFT, and SIT.

1. Prepulse inhibition

The effects of clozapine and VF on MIA-induced PPI deficits in mice are shown in Fig. 1.

We tested whether clozapine and VF treatment might be effective in preventing emergence of sensorimotor gating deficiency following MIA during adulthood. Sensorimotor gating was evaluated using the paradigm of PPI of acoustic startle reflex (ASR). Peripubertal clozapine and VF administration prevented the disruption of PPI in the offspring exposed to MIA ($p < 0.05$, Fig. 1). Clozapine and VF administration also decreased ASR levels in MIA offspring ($p < 0.05$, Fig. 1A).

Our results indicated that MIA significantly ($p < 0.05$) altered PPI level at prepulse stimulus levels of 6, 9, and 12 dB above background between MIA offspring and control offspring. VF treatment significantly ($p < 0.05$) elevated PPI trials scores in MIA offspring to levels found in control offspring (Fig. 1B). Poly I:C exposure to MIA affected ($p < 0.05$) the reactivity to mean percent PPI (Fig. 1C). These results suggest that clozapine and VF can prevent the development of sensorimotor gating deficiency in MIA offspring.

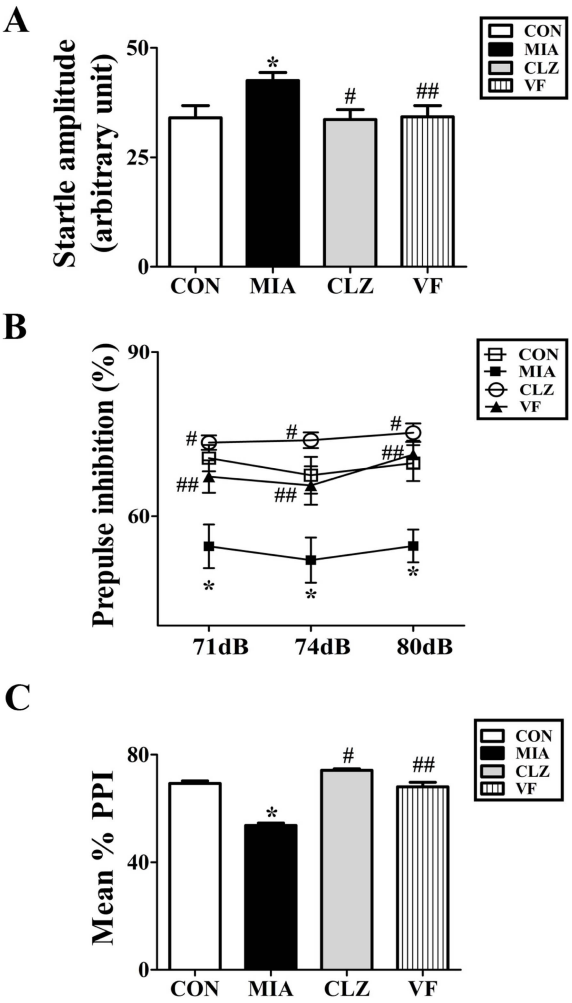

Fig. 1. Prepulse inhibition (PPI) was analyzed in adult male mice exposed to MIA or male mice derived from litters without MIA exposure. PPI is presented as a mean present PPI value in startle amplitude as a function of the magnitude of prepulse stimulus using the following formula; mean present PPI = 100 - [{(startle response for prepulse + pulse trial) / (startle response for pulse alone trial)} × 100]. Data are presented as means ± SEM (*$p < 0.05$ compared to CON, $^{\#,\#\#}p < 0.05$ compared to MIA). CON; offspring of non-MIA mice, MIA; offspring of MIA mice, CLZ; offspring of MIA mice treated with clozapine drug, VF; offspring of MIA mice treated with *Valeriana fauriei* extract.

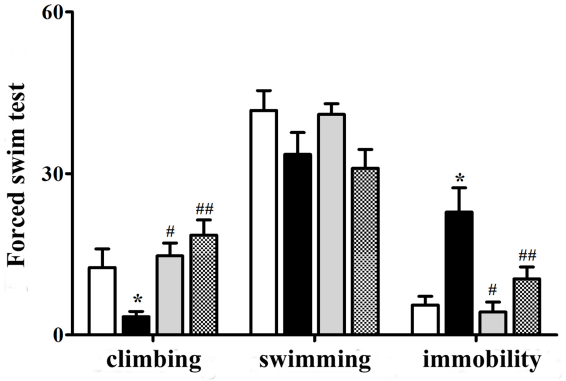

Fig. 2. Behavioral response in forced-swim test. Comparison between offspring of the CON and MIA groups. A decrease in climbing activity was observed. Data are presented as means ± SEM (*$p < 0.05$ compared to CON, $^{\#,\#\#}p < 0.05$ compared to MIA). CON; offspring of non-MIA mice, MIA; offspring of MIA mice, CLZ; offspring of MIA mice treated with clozapine drug, VF; offspring of MIA mice treated with *Valeriana fauriei* extract.

2. Forced swim test (FST)

We found significant differences in FST results among control, MIA, and VF-treated groups (Fig. 2). In particular, MIA offspring exhibited decrease in climbing but increase in immobility behaviors compared to control offspring ($p < 0.05$, Fig. 2). The changed behaviors were recovered after treatment with clozapine or VF ($p < 0.05$, Fig. 2).

3. Open-field test (OFT)

Control and MIA offspring were subjected to OFT for 20 min. The MIA group had a significantly decrease in the number of line crossings and duration of rearing behaviors. These scores were recovered to their normal levels after treatment with clozapine or VF ($p < 0.05$, Table 1).

The MIA group showed a significantly increase in the number of cage sniffing and immobility behaviors. These scores were also recovered to their normal level after treatment with clozapine or VF ($p < 0.05$, Table 1). However, the MIA offspring did not show significant difference in central entries, number of rearing behaviors, or the duration of cage sniffing compared to control offspring ($p > 0.05$, Table 1).

4. Social interaction test (SIT)

MIA also induced severe social deficits (Table 2). Scores of most aggressive behaviors (aggressive grooming the partner, biting the partner) during SIT were increased significantly in the MIA group compared to those in the control group. These scores were decreased to normal levels after treatment with clozapine or VF ($p < 0.05$, Table 2).

On the other hand, reductions in sniffing the partner

Table 1. Behavior of CON and MIA and clozapine treatment of MIA and *Valeriana fauriei* extract treatment of MIA in an open field test.

	CON	MIA	CLZ	VF
Central entered*	31.44 ± 3.35	15.56 ± 2.09	19.00 ± 2.84	20.44 ± 2.43
Line crossing*,#,##	09.78 ± 0.91	3.33 ± 0.44	7.33 ± 1.26	10.44 ± 1.33
Run (n)	0.11 ± 0.11	0.22 ± 0.22	0.22 ± 0.15	0.00 ± 0.00
Run (s)	0.22 ± 0.22	0.33 ± 0.33	0.56 ± 0.38	0.00 ± 0.00
Rear (n)*	117.56 ± 4.16	79.89 ± 6.79	79.33 ± 6.87	97.44 ± 8.42
Rear (s)*,##	245.67 ± 9.36	155.00 ± 15.03	153.22 ± 14.99	212.00 ± 19.48
Grooming (n)	6.89 ± 0.92	5.11 ± 1.01	5.00 ± 0.83	4.00 ± 0.62
Grooming (s)	42.56 ± 3.37	30.22 ± 6.76	31.89 ± 4.37	31.00 ± 5.86
Cage sniff (n)#,##	179.44 ± 7.04	166.11 ± 15.88	89.11 ± 9.23	115.56 ± 8.22
Cage sniff (s)	524.67 ± 25.43	447.00 ± 35.84	374.56 ± 53.45	429.44 ± 36.57
Immobile (n)*,#,##	3.22 ± 1.10	85.33 ± 10.16	19.11 ± 3.88	1.56 ± 0.53
Immobile (s)*,#,##	7.89 ± 2.75	238.44 ± 40.38	25.33 ± 4.94	5.33 ± 1.56

Data are presented as mean ± SEM, n; number of the behavior, s; duration measured in seconds, CON; non-Poly I:C-injection group, MIA; Poly I:C-injection group, CLZ; intraperitoneal injection with clozapine of Poly I:C-injection group, VF; administration with *Valeriana fauriei* extract of Poly I:C-injection group, *; between CON and MIA p-value < 0.05, #; between MIA and CLZ p-value < 0.05, ##; between MIA and VF p-value < 0.05.

Table 2. Behavior of CON and MIA and clozapine treatment of MIA and *Valeriana fauriei* extract treatment of MIA in a social interaction test.

	CON	MIA	CLZ	VF
Sniffing (n)*,#	93.83 ± 3.70	60.08 ± 3.37	74.92 ± 2.39	63.25 ± 2.43
Sniffing (s)*,#,##	152.25 ± 6.02	97.08 ± 4.61	154.00 ± 16.46	122.42 ± 7.42
Following (n)	3.50 ± 0.82	9.83 ± 5.60	7.08 ± 1.19	5.17 ± 0.82
Following (s)	9.08 ± 2.28	8.67 ± 1.55	14.92 ± 3.19	14.25 ± 2.34
Grooming the partner (n)##	1.08 ± 0.38	0.33 ± 0.19	0.50 ± 0.29	5.50 ± 2.14
Grooming the partner (s)##	6.33 ± 2.82	1.83 ± 1.09	2.42 ± 1.29	18.33 ± 7.10
Fight (n)	0.00 ± 0.00	0.33 ± 0.26	0.00 ± 0.00	0.00 ± 0.00
Fight (s)	0.00 ± 0.00	0.67 ± 0.51	0.00 ± 0.00	0.00 ± 0.00
Aggressive grooming (n)*,#,##	0.08 ± 0.08	1.08 ± 0.40	0.08 ± 0.08	0.00 ± 0.00
Aggressive grooming (s)*,##	0.17 ± 0.17	3.00 ± 1.20	0.58 ± 0.58	0.00 ± 0.00
Biting (n)*,#,##	2.58 ± 0.62	6.00 ± 1.36	0.17 ± 0.11	2.42 ± 0.96
Biting (s)#,##	10.00 ± 3.34	19.08 ± 5.12	0.58 ± 0.43	6.08 ± 2.39

Data are presented as mean ± SEM, n; number of the behavior, s; duration measured in seconds, CON; non-Poly I:C-injection group, MIA; Poly I:C-injection group, CLZ; intraperitoneal injection with clozapine of Poly I:C-injection group, VF; administration with *Valeriana fauriei* extract of Poly I:C-injection group, *; between CON and MIA p-value < 0.05, #; between MIA and CLZ p-value < 0.05,##; between MIA and VF p-value < 0.05.

and grooming the partner induced by MIA were rescued by treatment with VF. These results indicate that VF can ameliorate defective social interactions in MIA-induced animal model ($p < 0.05$, Table 2).

5. Western blot and immunohistochemistry

Our previous studies have shown that Lasp1 and Dpysl2 are regulated in prefrontal cortex in schizophrenia-like animal model (Joo *et al*., 2013; Lee *et al*., 2015).

To investigate MIA-induced downregulation of several neurodevelopmental proteins such as Lasp1, Dpysl2, and Nefm proteins, we performed Western blotting and immunohistochemical analyses (Fig. 3, 4, 5) of the mPFC areas from control, MIA, clozapine, and VF administered offspring brains. Western blot results revealed that the quantities of these three proteins in the mPFC area of the MIA group were significantly lower than those in the control group ($p < 0.05$, Fig. 3). These changes were restored by clozapine or VF treatment ($p < 0.05$, Fig. 3). Immunofluorescent-stained brain images revealed that

Dpysl2 and Nefm were differentially expressed among control, MIA, clozapine treated, and VF treated groups with significant differences in staining intensity value ($p < 0.05$, Fig. 4, 5).

The genus *Valeriana* contains over 250 species including subspecies such as *Valeriana fauriei* Briq. (VF) and *Valeriana officinalis* (Circosta *et al*., 2007). Several previous studies have shown that VF might improve sleep quality (Bent *et al*., 2006). The effects of VF on spatial memory have been demonstrated using a novel object recognition and water maze test (Nam *et al*., 2013).

VF also has antioxidant effects and antidepressant-like activity (Liu *et al*., 2012). Additionally, *in vitro* study has shown that *Valeriana officinalis* extract has neuroprotective properties against Aβ toxicity (Malva *et al*., 2004). In this study, we proposed that VF might be able to reverse schizophrenia-like behavior in MIA offspring using an animal model.

Using an epidemiologically motivated neurodevelopmental animal model of related psychiatric disorders such as depression and schizophrenia, previous studies showed that MIA in the form of viral-like acute phase response can trigger early/middle gestation (GD9) in mice, leading to long-lasting changes in behavior and protein expression (Meyer *et al*., 2010b). Impaired social interaction behavior was also observed in MIA offspring. This diminution in social interaction behaviors could reflect an increase in anxiety in MIA offspring (Bitanihirwe *et al*., 2010). The influence of MIA is consistent with previous reports on social interaction deficits emerged in adult mice after exposing to Poly I:C in middle gestation (Smith *et al*., 2007). In this current study, MIA-induced increases in aggressive behavior were restored by VF treatment. In addition, some behavioral patterns in FST and OFT were recovered by the treatment.

We found PPI deficits in the offspring of MIA treated by synthetic double-stranded RNA poly I:C. Braff *et al*. (2001) have shown deficits in the PPI of the startle response in both schizophrenic patients and animal models of this disorder (Braff *et al*., 2001). Such deficits in PPI are believed to reflect disruption in sensorimotor gating. We also found that exposure to MIA disrupted the gating as reflected by deficits in PPI and the deficit may be affected by VF treatment. Clozapine antipsychotic is well-known to be effective in treating positive and negative

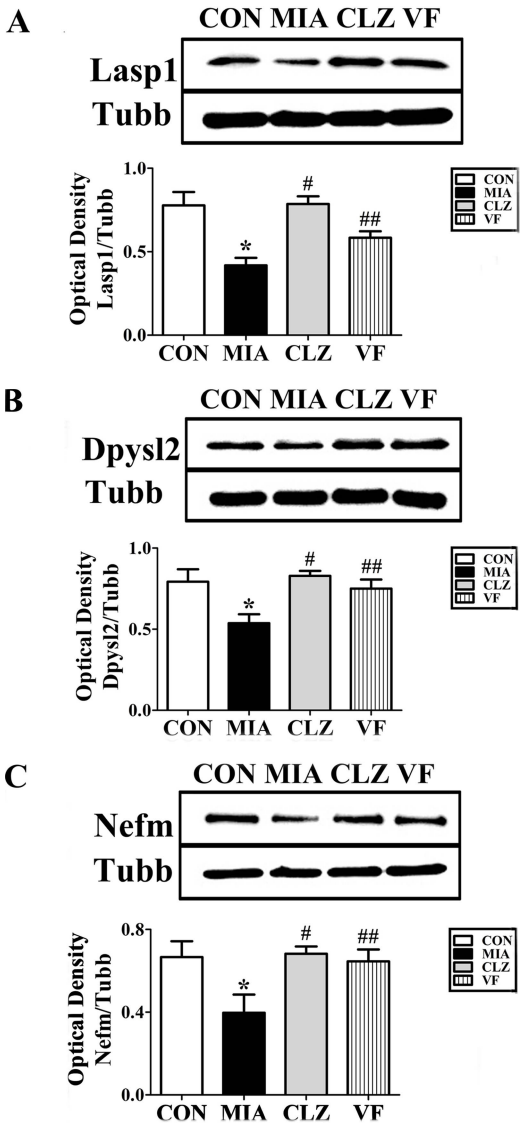

Fig. 3. Western blot analysis of Dpysl2, Lasp1, and Nefm expression in the brains of MIA-induced mice. (A); mice exposed to maternal immune activation (MIA) exhibited decreased Lasp1 expression in the medial prefrontal cortex. Lasp1 expression showed a significant difference in Lasp1 levels between MIA treated and VF-treated groups. (B); mice exposed to maternal immune activation (MIA) exhibited decreased Dpysl2 expression in the medial prefrontal cortex. Dpysl2 expression showed significant difference in Dpysl2 levels between MIA treated and VF-treated groups. (C); mice exposed to maternal immune activation (MIA) exhibited decreased Nefm expression in the medial prefrontal cortex. Nefm expression showed significant difference in Nefm levels between MIA treated and VF-treated groups ($^{*}p < 0.05$ compared to the CON group in the medial prefrontal cortex, $^{\#,\#\#}p < 0.05$ compared to MIA group in the medial prefrontal cortex). CON; offspring of non-MIA mice, MIA; offspring of MIA mice, CLZ; offspring of MIA mice treated with clozapine drug, VF; offspring of MIA mice treated with *Valeriana fauriei* extract.

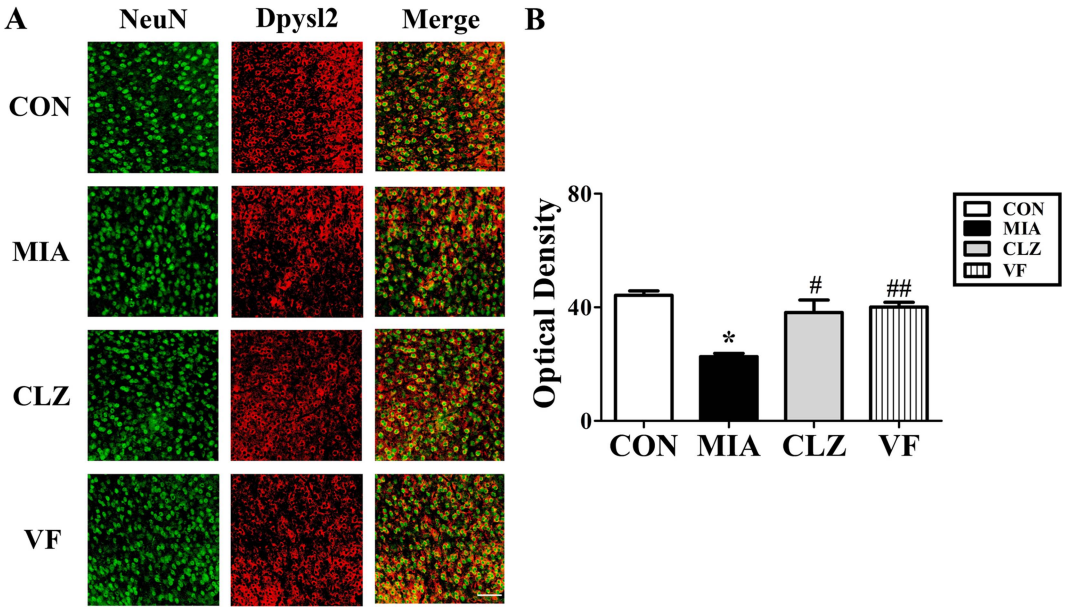

Fig. 4. Immunohistochemical analysis of Dpysl2 expression in the brains of MIA-induced mice. (A); confocal microscopic image showing immunofluorescent staining for Dpysl2 (anti-Dpysl2, red, Cy3) with NeuN in the medial prefrontal cortex. Fluorescent staining revealed a decrease of Dpysl2 in these regions. Scale bar, mPFC, 50 ㎛. (B); scatter gram in the graph indicates SEM (*$p < 0.05$ compared to CON, $^{\#,\#\#}p < 0.05$ compared to MIA). mPFC; medial frontal cortex, CON; offspring of non-MIA mice, MIA; offspring of MIA mice, CLZ; offspring of MIA mice treated with clozapine drug, VF; offspring of MIA mice treated with *Valeriana fauriei* extract, SEM; Standard error of the mean.

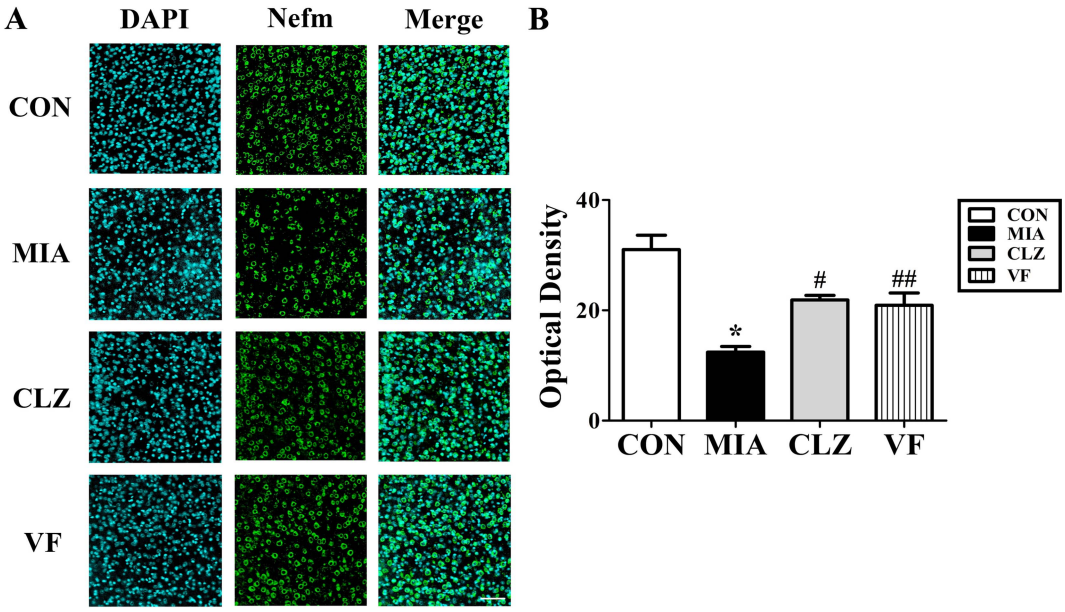

Fig. 5. Immunohistochemical analysis of Nefm expression in the brains of MIA-induced mice. (A); confocal microscopic image showing immunofluorescent staining for Nefm (anti-Nefm, green, FITC) with DAPI (blue) in the medial prefrontal cortex. Fluorescent staining revealed a decrease of Dpysl2 in these regions. Scale bar, mPFC, 50 ㎛. (B); the scatter gram in the graph indicates SEM (*$p < 0.05$ compared to CON, $^{\#,\#\#}p < 0.05$ compared to MIA). mPFC; medial frontal cortex, CON; offspring of non-MIA mice, MIA; offspring of MIA mice, CLZ; offspring of MIA mice treated with clozapine drug, VF; offspring of MIA mice treated with *Valeriana fauriei* extract, SEM; Standard error of the mean.

symptoms of schizophrenia (Manschreck *et al*., 1999) or reverse PPI disruption induced by MIA in offspring (Meyer *et al*., 2010a). The efficacy of clozapine has been attributed to its action on several different mPFC neurotransmitter systems (Johnston-Wilson *et al*., 2000).

In the present study, the typical antipsychotic clozapine was effective in restoring PPI deficits observed in MIA offspring treated with Poly I:C during pregnancy.

In this study, we investigated the levels of two proteins; Dpysl2 and neurofilament protein. The decrease of mPFC in neurofilament and Dpysl2 proteins due to MIA was affected by VF treatment. Dpysl2, also known as collapsin response mediator protein 2, can regulate axonal outgrowth by promoting microtubule assembly, vesicle trafficking, and synaptic physiology (Lin *et al*., 2011). The expression of Dpysl2 in humans has been reported to be decreased in the brains of patients with schizophrenia (Johnston-Wilson *et al*., 2000).

Neurofilament protein may also play a role in intracellular transport to axons and dendrites. It forms part of the axon skeleton and functionally maintains neuronal caliber (Cassereau *et al*., 2013). Our previous study has shown that Dpysl2 and neurofilament protein levels are decreased in stress-induced schizophrenia-like rat offspring but recovered by VF treatment (Lee *et al*., 2016a). These findings suggest that changes in the expression of neurodevelopmental proteins such as neurofilament proteins and Dpysl2 caused by MIA might have enduring effects on axonal outgrowth and synaptic function in MIA offspring.

Our results showed that maternal administration of Poly I:C into MIA caused abnormality of behavioral pattern, deficits in sensory-motor gating, and aggressive behavior in SIT. However, these effects could be improved by oral administration extract of VF. Some behavioral patterns in FST and OFT for the analysis of depressive behaviors were recovered by treatment with VF.

In addition, MIA-induced decrease in the expression levels of neurofilament and Dpsyl2 was restored to normal levels after treatment with VF. The present study provided valuable data regarding additional role of VF in addressing the pathogenesis of psychiatric disorders such as schizophrenia. However, further research is needed to characterize the pharmacological functions of VF using cellular and animal models.

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