Research Article

Postnatal BDNF Expression Profiles in Prefrontal Cortex and Hippocampus of a Rat Schizophrenia Model Induced by MK-801 Administration

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Neonatal blockade of N-methyl-D-aspartic acid (NMDA) receptors represents one of experimental animal models for schizophrenia. This study is to investigate the long-term brain-derived neurotrophic factor (BDNF) expression profiles in different regions and correlation with "schizophrenia-like" behaviors in the adolescence and adult of this rat model. The NMDA receptor antagonist MK801 was administered to female Sprague-Dawley rats on postnatal days (PND) 5 through 14. Open-field test was performed on PND 42, and PND 77 to examine the validity of the current model. BDNF protein levels in hippocampus and prefrontal cortex (PFC) were analyzed on PND 15, PND 42, and PND 77. Results showed that neonatal challenge with MK-801 persistently elevated locomotor activity as well as BDNF expression; the alterations in BDNF expression varied at different developing stages and among brain regions. However, these findings provide neurochemical evidence that the blockade of NMDA receptors during brain development results in long-lasting alterations in BDNF expression and might contribute to neurobehavioral pathology of the present animal model for schizophrenia. Further study in the mechanisms and roles of the BDNF may lead to better understanding of the pathophysiology of schizophrenia.

1. Introduction

Schizophrenia is a complex and severe brain disorder with poorly defined etiology and pathophysiology. Many diverse lines of evidence indicate that schizophrenia is a neurodevelopment disorder; early-life adverse events such as prenatal stress, obstetric complications, malnutrition, and viral infection may interrupt the normal brain development and increase the susceptibility of schizophrenia later in life [1–4]. Neurotrophic molecules are believed to be critical in the development and function maintenance of cortical neurons and synapses, such as brain-derived neurotrophic factor (BDNF) [5]. BDNF is a key member of the neurotrophin family widely expressed in the brain [6, 7]; it plays an important and unique role in regulating a wide repertoire of functions at different development stages, including neuronal survival, migration, phenotypic differentiation, and axonal and dendritic growth in neonatal individuals [8, 9], as well as synaptic plasticity and behavior in adulthood [10–12].

Several lines of evidence have suggested the involvement of BDNF in pathophysiology of schizophrenia in both human and animal studies [13–15]. However, knowledge in understanding of BDNF in pathophysiology of schizophrenia remains limited and poorly defined. One of key pitfalls of schizophrenia research in animal studies is that all experiments usually focused only on a certain period during development. Therefore, it is fundamentally and clinically significant to investigate long-term changes of BDNF in animal models of schizophrenia in a development scheme.

Among a number of schizophrenia animal models, repeated administration of dizocilpine maleate (MK-801, a noncompetitive NMDA receptor antagonist) during neonatal period may be a suitable animal model to explore certain remaining issues. This is because the paradigm of this model has been shown to produce "schizophrenia-like" behaviors weeks after administration [16], a potential advantage over other models to study long-lasting effects in a development scheme. The aim of the present study is to investigate the

long-term BDNF expression profiles in different regions and correlation with "schizophrenia-like" behaviors in the adolescence and adult of this rat model.

2. Materials and Methods

2.1. Animals. Timed-pregnant Sprague-Dawley rats were purchased from the Department of Laboratory Animal Science, Peking University Health Science Center. Female pups were collected and then cross-fostered to one of the lactating dams within 24 h of parturition, which was designated as Postnatal day 0 (PND 0). Animals were housed on a 12 : 12 light/dark cycle (lights on 08:00 hour) with ad libitum access to food and water. All procedures were performed in accordance with the National Institute of Health Guide for Use and Care of Laboratory Animals and were approved by the Peking University Committee on Animal Care and Use.

On PND 5, the pups from each litter were randomly assigned to the MK-801 or saline groups, which were injected subcutaneously with MK-801 or saline for 10 consecutive days. All groups were sex balanced. To avoid potential influence on BDNF levels by behavioral testing, BDNF assessment and behavioral testing were performed on different rats. For BDNF assessment, rats were sacrificed and assessed for BDNF levels 24 hours (PND 15) and 4 weeks (PND 42) after the last injection. For behavioral testing, all animals were tested for locomotor activity at PND 42 and PND 77. Pups (except those sacrificed on PND 15) were weaned on PND 22 and then housed 4 per cage.

2.2. Drug Treatment. MK-801 was obtained from Sigma (St. Louis, MO, USA) and dissolved in saline (0.9% NaCl). Based on our preliminary experiment, a dose of 0.25 mg/kg per injection of MK-801 was used in the present study. All rats received injections twice daily beginning at 9:00 and 15:00, respectively.

2.3. Locomotor Activity. Because locomotor activity has been thought to be an indicator of "schizophrenia-like" behavior [17–19], to test the face validity of the MK-801 administration in mimicking schizophrenia, we measured locomotor activity. Two tests on PD 42 and PD 77 for each animal were conducted, of which the procedures were adapted from our previous work [20]. Briefly, locomotor activity was measured by an automated video tracking system with four activity chambers (DigBehv-LM4, Shanghai Jiliang Software Technology Co. Ltd, China). The chambers were made of black iron (45 cm × 45 cm × 70 cm, length × width × height). A monochrome video camera was mounted at the top of each chamber. All of the chambers were connected to a PC which recorded the locomotion of rat. The video documents (stored in the computer) were analyzed by the DigBehv analysis software. The analysis resulted in a track record, and locomotor activity was expressed as a total distance traveled for a predetermined period of time. On the testing day, rats were kept in testing room at least for 60 minutes before placing animals in the activity chambers. The locomotion was recorded for 60 minutes each time.
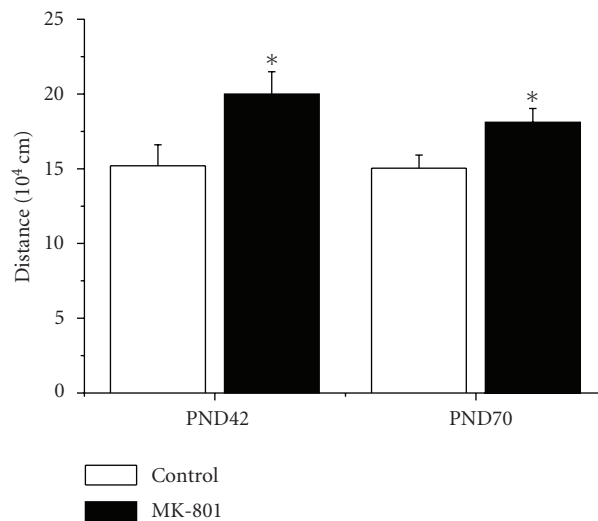

FIGURE 1: Neonatal MK-801 treatment increased the total locomotor activity on both PND 42 and PND 77. *, $P < .05$.

2.4. Measurement of BDNF Protein Levels. The BDNF Emax ImmunoAssay system (Promega) was used to measure BDNF protein levels in each brain region according to the manufacturer's protocol. Briefly, on PN15 (24 hours after the last injection) and PN42 (4 weeks after the last injection), brain tissues of hippocampus and PFC were immediately dissected out and frozen on dry ice. Then the dissected brain tissues were homogenized in lysis buffer (137 mm NaCl, 20 mm Tris, 1% NP-40 detergent, 10% glycerol, 1 mm methylphenylsulphonylfluoride, 10 mg/mL aprotinin, 1 mg/mL leupeptin, and 0.5 mm sodium orthovanadate). Homogenates were centrifuged at 10,000 rpm for 10 min in a cold room, and supernatants were used for ELISA analysis. A standard curve was made using serial dilutions of known amounts of BDNF ranging from 0 to 500 pg/mL. Results for duplicate experiments were averaged, and BDNF values were corrected for the amount of protein in each sample. The sensitivity of the assay was within the range reported by the above-mentioned Promega kit and the interassay variation was less than 10%.

2.5. Statistics. All data were expressed as means ± S.E.M. Statistical differences between groups were determined by independent samples t-test. A probability level of $P < .05$ was considered to be statistically significant. Statistical analyses were performed using SPSS 17 for windows.

3. Results

3.1. Locomotor Activity Were Elevated on PND 42 and PND 77. MK-801 treated rats exhibited significantly increased locomotor activity compared with saline-treated controls on both PND 42 ($t = -2.63$, $df = 17$, and $P = .018$) and PND 77 ($t = -2.43$, $df = 20$, and $P = .025$) (Figure 1).

3.2. Changes of BDNF Protein Expression in the PFC and Hippocampus on PND 15, PND 42, and PND 77. Protein content of BDNF was significantly increased, but with
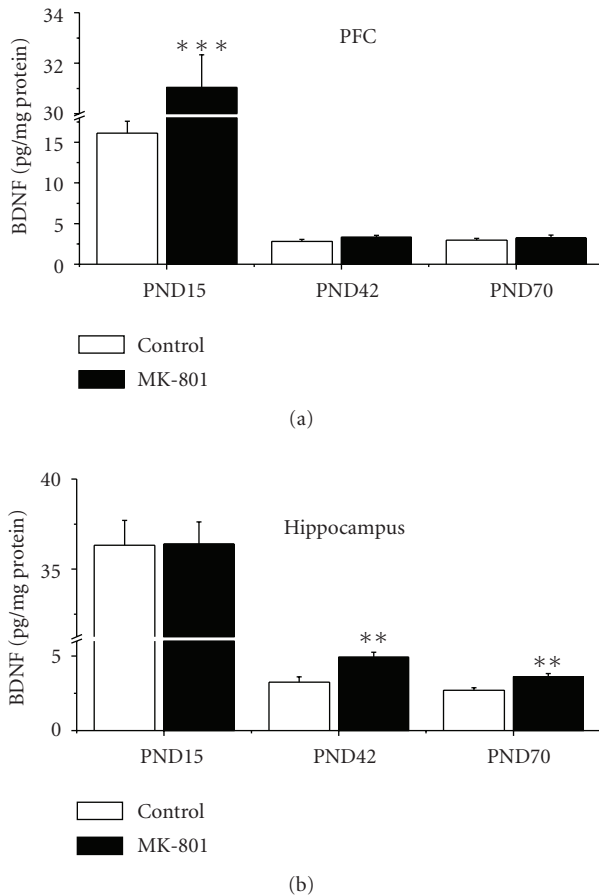
(a)

(b)

Figure 2: The influence of neonatal MK-801 treatment on BDNF concentrations in (a) PFC and (b) hippocampus on different postnatal days. *, **, *** $P < .05$, $P < .01$, $P < .001$ respectively.

hippocampal BDNF upregulation was concomitant with the behavioral abnormality of the animals. The above findings altogether indicate that altered BDNF expression in this experimental animal model for schizophrenia may be correlated with the development of schizophrenia-like behavior and pathophysiology.

It has been demonstrated that Schizophrenia is usually diagnosed during adolescence, a period experiencing dramatic changes in behaviors [21]; interestingly BDNF expression in human brain changes during development and maturation [22]. Therefore it is necessary and important to include different time points in the current study. We chose PFC and hippocampus in the current study, because these two brain areas are most implicated in schizophrenia [23, 24]. Abnormal BDNF expression has been observed in these two areas [15], and conditional knockout of BDNF in the forebrain induced impaired locomotor activity in rodents [25].

Locomotor hyperactivity is routinely used as indicator of "schizophrenia-like" behavior [17–19] and has been found in adolescent or adult animals experienced neonatal NMDA receptor blockade [16, 26]. Our finding showed the feasibility of assessing BDNF levels in such a developmental rat model long after the drug administration. On PND 15, the BDNF expression increased in PFC but not in hippocampus. However, on PND 42, the upregulated BDNF levels in PFC returned to normal level, while the BDNF expression in hippocampus significantly increased and sustained from PND 42 to PND 77. We did not assess the locomotor activity on PD15, since the preweaning animal is not suitable for behavioral assessment of schizophrenia model.

Our findings are consistent with some preclinical and clinical studies. A previous clinical observation showed that BDNF protein was increased in hippocampus and unchanged in PFC of schizophrenia patients [27]. Since the above tested brain samples of all patients received antipsychotic treatment before death, the association of the altered BDNF expressions with schizophrenia pathology or antipsychotic drug effects remains unknown. Furthermore, it is not known whether the expression of BDNF in schizophrenia patients changes at different ages. The current study demonstrates that the BDNF levels in MK-801 treated rats persisted from PND 42 to PND 77, suggesting that the abnormal expression patterns of BDNF observed in schizophrenia patients might not be affected by age during adolescence and early adulthood. Interestingly, the abnormal BDNF expression occurred between PND 15 and PND 42, adolescence in rats. This is consistent with clinical observations that schizophrenia is usually diagnosed at late adolescence or early adulthood. This plausible coincidence of schizophrenia age onset and the emergence of the BDNF increase hints at the association of BDNF expression and the etiology of schizophrenia. Additionally, there is evidence that BDNF protein levels may cause changes in strength of synaptic transmission in hippocampus [28], and upregulated BDNF levels in hippocampus can induce other schizophrenia-like behavior [29]. Thus, it is possible that the locomotor hyperactivity is related with the increased BDNF in hippocampus but not PFC in the current study.

different patterns in PFC and hippocampus. In PFC, the BDNF expression was significantly upregulated in MK-801 treated rats ($t = -6.19$, $df = 16$, and $P < .001$) at early time point of PND 15. However, this early increase of BDNF expression was only transient, and it went back to normal control baseline on PND 42 ($t = -1.19$, $df = 19$, and $P > .05$) and PND 77 ($t = -0.54$, $df = 17$, and $P > .05$) (Figure 2(a)). In contrast to the developmental pattern in PFC, BDNF expression in the hippocampus was not influenced by neonatal MK-801 administration ($t = -0.30$, $df = 16$, and $P > .05$) on PND 15. However, there was a significant late increase of BDNF expression on PND 42 ($t = -3.89$, $df = 16.9$, and $P = .001$) and PND 77 ($t = -3.28$, $df = 17$, and $P = .01$) (Figure 2(b)).

4. Discussion

The major findings of present study are (1) exposure of the developing brain to MK-801, neonatal blockade of NMDA receptor, resulted in the locomotor hyperactivity in adolescence and in adult of rats; (2) BDNF levels in brain tissues were affected by neonatal MK-801 administration with patterns of age-dependent, long-lasting, and brain regional specificity; (3) the postpubertal emergence of the

The increase of BDNF levels at PFC is probably a protective response to MK-801 administration induced cytotoxic insults [26, 30]. The fact that hippocampus is spared from acute responses to MK-801 treatment suggests that the alterations of BDNF in adolescence and adulthood were not remnants of acute responses. Therefore, the BDNF changes at adolescence and adulthood may have different roles and mechanisms with that of neonatal stage, which needs further investigations. It could be seen from the current study that the alterations of BDNF expression and locomotor activity both persisted from PND 42 to PND 77. The plausible association between changes in behavioral performance and BDNF expression indicates that regulation of BDNF expression may be related with pathophysiology of schizophrenia.

We are aware that there are several limitations of the current study. It has been reported that BDNF levels in serum are abnormal in patients with schizophrenia [31, 32]; it would be interesting and important to have information on circulation, BDNF levels in our future studies. The correlations between behavioral deficits and BDNF alterations are incompletely understood from this study; a more thorough behavioral profile needs to be performed in the future. BDNF protein levels alone may not give a complete account of BDNF/trk B signaling, and the function of BDNF/trk B signaling may vary among subregions of hippocampus [33]. mRNA and protein may have different developing trajectories, and different transcripts of BDNF may have different development trajectories [34]; more studies for different mRNA transcripts of BDNF need to be carried out. There may be multiple neurotrophic molecules and signaling pathways involved in this model; interaction and a network regulation need to be aware, and investigated as well.

In summary, our present study demonstrates that there are sustained behavioural deficit and BDNF alterations in the rat model of schizophrenia induced by neonatal MK-801 administration. These findings suggest that altered expression of BDNF might attribute to schizophrenia and provide evidence that the patterns of abnormal BDNF expression may vary at different developing stages. Further experiment needs to elucidate the mechanisms underlying the age-associated changes of BDNF and the potential roles of BDNF on abnormal behavior, which may lead to a better understanding of the pathophysiology of schizophrenia.

Acknowledgments

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