Please cite this article in press as: Li J-T et al. Long-term effects of neonatal exposure to MK-801 on recognition memory and excitatory–inhibitory balance in rat hippocampus. Neuroscience (2015), http://dx.doi.org/10.1016/j.neuroscience.2015.09.003

Neuroscience xxx (2015) xxx–xxx

LONG-TERM EFFECTS OF NEONATAL EXPOSURE TO MK-801 ON RECOGNITION MEMORY AND EXCITATORY–INHIBITORY BALANCE IN RAT HIPPOCAMPUS

J.-T. LI,[a†] Y.-Y. ZHAO,[b†] H.-L. WANG,[a] X.-D. WANG,[c] Y.-A. SU[a*] AND T.-M. SI[a*]

[a] *National Clinical Research Center for Mental Disorders (Peking University Sixth Hospital/Institute of Mental Health) and the Key Laboratory of Mental Health, Ministry of Health (Peking University), Beijing 100191, China*

[b] *Depression Treatment Center, Beijing Anding Hospital of Capital Medical University, Beijing 100088, China*

[c] *Department of Neurobiology, Key Laboratory of Medical Neurobiology of Ministry of Health of China, Zhejiang Province Key Laboratory of Neurobiology, Zhejiang University School of Medicine, 310058 Hangzhou, China*

Abstract—Blockade of the N-methyl-D-aspartate receptors (NMDARs) during the neonatal period has been reported to induce long-term behavioral and neurochemical alterations that are relevant to schizophrenia. In this study, we examined the effects of such treatment on recognition memory and hippocampal excitatory–inhibitory (E/I) balance in both adolescence and adulthood. After exposure to the NMDAR antagonist, MK-801, at postnatal days (PND) 5–14, male Sprague–Dawley rats were tested for object and object-in-context recognition memory during adolescence (PND 35) and adulthood (PND 63). The parvalbumin-positive (PV +) γ-aminobutyric acid (GABA)-ergic interneurons and presynaptic markers for excitatory and inhibitory neurons, vesicular glutamate transporter-1 (VGLUT1) and vesicular GABA transporter (VGAT) were examined in the hippocampus to reflect the E/I balance. We found that rats receiving MK-801 treatment showed deficits of recognition memory, reduction in PV+ cell counts and upregulation of the VGLUT1/VGAT ratio in both adolescence and adulthood. Notably, the changes of the VGLUT1/VGAT ratio at the two time points exhibited distinct mechanisms. These results parallel findings of hippocampal abnormalities in schizophrenia and lend support to the usefulness of neonatal NMDAR blockade as a potential neurodevelopmental model for the disease. © 2015 Published by Elsevier Ltd. on behalf of IBRO.

Key words: cognition, parvalbumin, schizophrenia, VGLUT1, VGAT.

INTRODUCTION

Perinatal N-methyl-D-aspartate receptor (NMDAR) antagonist treatment in rodents offers a useful tool to uncover the etiology of schizophrenia, since this model is based on both the neurodevelopmental (Kirch, 1993; McGrath et al., 2003; Rapoport et al., 2005; Rapoport et al., 2012) and NMDAR hypofunction (Javitt and Zukin, 1991; Krystal et al., 1994) hypotheses of the disease. Indeed, it has been reported that such treatment causes long-term behavioral and neurochemical alterations that are relevant to the symptoms of schizophrenia (du Bois and Huang, 2007).

The hippocampus has long been implicated in the pathophysiology of schizophrenia. Hippocampal abnormalities in schizophrenic patients have been reported in domains including neuroanatomy, neurodevelopment, biochemistry and genetics (for review, see (Boyer et al., 2007). Recently, postmortem studies have shown substantial deficits of the γ-aminobutyric acid (GABA)-ergic interneurons, particularly the parvalbumin-positive (PV+) cells, in the hippocampus (Zhang and Reynolds, 2002). This finding has been replicated in various animal models, such as isolation rearing (Harte et al., 2007) and chronic NMDAR antagonism in juvenile and adult rats (Abdul-Monim et al., 2007; Braun et al., 2007). Effects of neonatal NMDAR blockade on the PV+ interneurons have been investigated in adult animals, and conflicting results were obtained. While some studies found that a single injection of MK-801 on postnatal day 7 (PND 7) did not change PV + cell density in the hippocampus or any of its subregions (Wang et al., 2008; Gilabert-Juan et al., 2013), others reported the decrease of this cell type in the hippocampus as a whole or in some, but not all, subregions (Nakatani-Pawlak et al., 2009; Uehara et al., 2012a,b).

Considering that PV+ interneurons play an important role in regulating pyramidal neuron activity by exerting strong inhibitory control (DeFelipe et al., 1989), the reduced density of these neurons in the hippocampus may be accompanied by altered excitatory and inhibitory (E/I) neurotransmission at the synaptic level. Indeed, postmortem studies indicate that the presynaptic protein genes, complexin I and II mRNA, markers for excitatory and inhibitory neurons respectively, were reduced in

*Corresponding authors. Address: Department of Psychopharmacology, Institute of Mental Health, Peking University, Beijing 100191, China. Tel: +86-10-62352880; fax: +86-10-62027314.
E-mail addresses: suyunai@163.com (Y.-A. Su), si.tian-mei@163.com (T.-M. Si).
† These authors contribute equally to the paper.

Abbreviations: DG, dentate gyrus; E/I, excitatory–inhibitory; NMDAR, N-methyl-D-aspartate receptor; NS, normal saline; PCP, phencyclidine; PND, postnatal days; PV+, parvalbumin-positive; VGAT, vesicular GABA transporter; VGLUT1, vesicular glutamate transporter-1.

http://dx.doi.org/10.1016/j.neuroscience.2015.09.003
0306-4522/

schizophrenia (Harrison and Eastwood, 1998). Here, we focus on another pair of selective presynaptic markers for excitatory and inhibitory neurons, vesicular glutamate transporter-1 (VGLUT1) and vesicular GABA transporter (VGAT). Localized in the vesicles of excitatory and inhibitory synapses, these transporters are responsible for the reuptake of glutamate and GABA, respectively, into vesicles of presynaptic terminals for their subsequent release into the synaptic cleft (Eiden, 2000), and contribute significantly to the regulation of the E/I neurotransmission by impacting vesicle filling and quantal size (Wojcik et al., 2004; Wilson et al., 2005). Therefore, examining their expression levels could inform us of the E/I neurotransmission at the synaptic level, but whether and how they would be affected by neonatal NMDAR antagonism has not been investigated.

Cognitive impairments have been proposed as a core feature of schizophrenia (Ranganath et al., 2008). We and others have shown that neonatal NMDAR blockade causes long-term cognitive deficits that are relevant to schizophrenia (Stefani and Moghaddam, 2005; Su et al., 2011, 2014), including spatial learning and working memory. In addition, visual learning and memory is one of the seven cognitive domains identified as commonly deficient in schizophrenia patients according to the Measurement and Treatment Research to Improve Cognition in Schizophrenia initiative (Green et al., 2004). Recognition memory tasks in rodents that encompass an animal's natural exploratory tendency toward novelty have been suggested as a useful tool for the investigation of this cognitive domain (Young et al., 2009). In this study, we performed two types of recognition memory tasks, object recognition and object-in-context recognition tasks, to evaluate the effects of neonatal NMDAR antagonism on item and associative recognition memory (Li et al., 2011).

Up to date, the majority of studies investigating long-term effects of neonatal NMDAR blockade focused on adulthood, and little is known whether the deleterious effects would appear earlier, for instance, in adolescence. Since psychosis typically has its first onset during adolescence, evaluating whether the model of neonatal NMDAR antagonist treatment could induce at least some schizophrenia-relevant deficits in this period would add further evidence to demonstrate its validity in mimicking the time course of schizophrenia and has important implications for early pharmacological interventions. Therefore, in the present study we examined, both in adolescent and adult rats, how neonatal NMDAR blockade would affect recognition memory, hippocampal PV+ interneurons and E/I balance at the synaptic level.

EXPERIMENTAL PROCEDURES

Animals

Timed pregnant Sprague–Dawley rats at gestation day 18 were purchased from the Department of Laboratory Animal Science, Peking University Health Science Center. The day of birth was considered as PND 0. Pups were weaned on PND 21 and housed with four per cage. Only male rats were used as experimental subjects. All animals were maintained at 12-h light/dark cycle (lights on at 08:00 am) in a temperature and humidity-controlled room (23 ± 1 °C, 45–55%) with free access to food and water. All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Peking University Committee on Animal Care and Use.

Drugs and pharmacological procedures

MK-801 [(5R, 10S)-(+)-5-methyl-10, 11-dihydro-5H-dibenzo [a, d] cyclohepten-5, 10-imine hydrogen maleate] was purchased from Sigma (St. Louis, MO, USA). Based on our previous experiments (Guo et al., 2010; Zhao et al., 2013; Su et al., 2014), we used the dose of 0.25 mg/kg, which has been shown to be the threshold dose of MK-801 for inducing apoptotic damage (Ikonomidou et al., 1999). On PND 5, male pups were randomly divided to normal saline (NS) and MK-801 groups, and received a subcutaneous injection of either saline or MK-801 in a final volume of 5 ml/kg, twice daily, on PND 5–14. The two injections occurred at 09:00 and 16:00 h. Three animal cohorts were used in this study, and the timing of various procedures in different cohorts is shown in Fig. 1. Body weights were recorded every day during the treatment period and every week from drug cessation to PND 63.

Behavioral procedures

The novel object and object-in-context recognition tasks were conducted in two separate age cohorts (PND 35–38, 19 animals in total with nine in the NS group and 10 in the MK-801 group; PND 63–66, 16 animals in total with eight per group). The tasks were performed as previously reported (Li et al., 2013) with only minor modifications. Two consecutive days before testing, animals were habituated to the empty testing environments with no objects inside for 10 min per day. This allowed animals to familiarize with the testing environments so that environmental exploration did not interfere with object interaction. Then came two testing days, one for the novel object recognition task and one for the object-in-context recognition task.

The novel object recognition task (NORT) consisted of a 10-min sample trial and a 3-min test trial. Two types of metal-made objects (in duplicate, 7–8 cm high) were used: cubes and cylinders. On the testing day, a 10-min sample trial was first given, during which the animal was placed in a black plexiglass chamber (40 × 40 × 65 cm) to freely explore two identical objects (either cubes or cylinders). The test trial came 90 min later, during which one of the familiar objects was replaced by a novel object and the animal was re-introduced into the chamber to explore the two objects for 3 min. The duration of the test trial, i.e. 3 min, has been shown to be a sensitive time period in object recognition task. Longer test duration may decrease the object recognition effect because the new object becomes increasingly familiar as time passes (Eacott and Norman, 2004).

Please cite this article in press as: Li J-T et al. Long-term effects of neonatal exposure to MK-801 on recognition memory and excitatory–inhibitory balance in rat hippocampus. Neuroscience (2015), http://dx.doi.org/10.1016/j.neuroscience.2015.09.003