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Research article

Effects of chronic forced-swim stress on behavioral properties in rats with neonatal repeated MK-801 treatment

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ABSTRACT

The two-hit hypothesis has been used to explain the onset mechanism of schizophrenia. It assumes that predisposition to schizophrenia is originally attributed to vulnerability in the brain which stems from genetic or early developmental factors, and that onset is triggered by exposure to later detrimental factors such as stress in adolescence or adulthood. Based on this hypothesis, the present study examined whether rats that had received neonatal repeated treatment with an *N*-methyl-D-aspartate (NMDA) receptor antagonist (MK-801), an animal model of schizophrenia, were vulnerable to chronic stress. Rats were treated with MK-801 (0.2 mg/kg) or saline twice daily on postnatal days 7–20, and animals in the stress subgroups were subjected to 20 days (5 days/week × 4 weeks) of forced-swim stress in adulthood. Following this, behavioral tests (prepulse inhibition, spontaneous alternation, open-field, and forced-swim tests) were carried out. The results indicate that neonatal repeated MK-801 treatment in rats inhibits an increase in immobility in the forced-swim test after they have experienced chronic forced-swim stress. This suggests that rats that have undergone chronic neonatal repeated NMDA receptor blockade could have a reduced ability to habituate or adapt to a stressful situation, and supports the hypothesis that these rats are sensitive or vulnerable to stress.

1. Introduction

N-methyl-D-aspartate (NMDA) receptors, a subtype of ionotropic glutamate receptors, have been implicated in many neural processes. Therefore, treatment with NMDA antagonists impairs numerous neural and mental functions. For example, many studies have shown that NMDA receptors are involved in long-term potentiation, which is believed to be one of the physiological bases for learning and/or memory (Abraham and Mason, 1988; Harris et al., 1984; Morris, 1989; Morris et al., 1986; Morris et al., 1989), and that NMDA receptor antagonists induce learning and/or memory impairments (Kawabe et al., 1998a; Kawabe et al., 1998b; Morris, 1989; Morris et al., 1986; Morris et al., 1989; Yoshihara and Ichitani, 2004). Furthermore, it is also well known that NMDA receptor antagonists such as phencyclidine (PCP) and ketamine induce positive and negative schizophrenia-like symptoms in humans and animals (Bubeníková-Valešová et al., 2008; Javitt and Zukin, 1991).

In addition to these findings based mainly on treatment with NMDA receptor antagonists in adulthood, it is also frequently reported that repeated treatment with antagonists such as PCP and MK-801 (dizocilpine; 5-methyl-10,11-dihydro-5H-dibenzo[*a*,*d*]cyclohepten-5,10-imine) during the early developmental stage can cause long-term alterations of anatomical, neurochemical, neurophysiological, behavioral, and other parameters in rats and mice (for example, Facchinetti et al., 1993; Facchinetti et al., 1994; Gorter et al., 1992a; Gorter and de Bruin, 1992; Gorter et al., 1991; Gorter et al., 1992b; Kawabe et al., 2007; Kawabe and Miyamoto, 2008; Nakatani-Pawlak et al., 2009; Niikura et al., 2015; Sircar, 2003; Stefani and Moghaddam, 2005; Wang et al., 2003). Additionally, since blockade of NMDA receptors in early developmental stages induces neural degeneration, and impairs normal development of the neural circuits and the brain (Beninger et al., 2002; Facchinetti et al., 1993; Facchinetti et al., 1994; Ikonomidou et al., 1999; Kawabe and Miyamoto, 2008; Nakatani-Pawlak et al., 2009; O'Donoghue et al., 1993; Wang et al., 2004; Wang et al., 2003), rats or mice that have received this treatment are used as animal models which adhere to the neurodevelopmental hypothesis of schizophrenia. This hypothesis assumes that maldevelopment of the brain, which is caused by genetic defects, viral infection, obstetric problems and other detrimental factors in early developmental stages, contributes to the onset of schizophrenia (Lewis and Levitt, 2002; Weinberger, 1987). Our previous studies showed that neonatal repeated treatment with competitive (CGS 19755; *cis*-4-phosphonomethyl-2-piperidine carboxylic acid)

Abbreviations: ANOVA, analysis of variance; DNMTP, delayed nonmatching-to-position; HPA, hypothalamic–pituitary–adrenal; NMDA, *N*-methyl-D-aspartate; PCP, phencyclidine; PND, postnatal day; PPI, prepulse inhibition; SA, spontaneous alternation; SAL, saline

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and noncompetitive (MK-801) NMDA receptor antagonists impaired spatial working memory in the radial-arm maze or the delayed non-matching-to-position (DNMTP) tasks (Kawabe et al., 2007; Kawabe and Miyamoto, 2008). Since working memory is severely impaired in schizophrenic patients (Goldman-Rakic, 1994; Manoach, 2003), these results validate the use of animals with neonatal NMDA receptor blockade as a preclinical model of schizophrenia.

In addition to genetic and early developmental factors, stress is one of the risk factors associated with a variety of mental disorders. The two-hit hypothesis of schizophrenia assumes that genetic or early developmental defects (first hit) are potential factors in the onset of schizophrenia, and that later detrimental factors in adolescence or adulthood (second hit) trigger this onset (Bayer et al., 1999; Feigenson et al., 2014; Maynard et al., 2001). Since stress is considered to be a major second hit factor, it is valuable to examine whether animal models of schizophrenia are vulnerable to stress. The present study examined whether rats subjected to neonatal repeated treatment with an NMDA receptor antagonist (MK-801), an animal model of schizophrenia, had stress-vulnerability as assessed by several behavioral or cognitive measures; sensorimotor gating, working memory, locomotor activity, and stress coping. These behavioral parameters were tested after chronic forced-swim stress had been applied in adulthood. Forced-swim stress is commonly used in stress studies of rats and mice, and it has been frequently reported to induce stress-related endocrinal, neural, and behavioral alterations (for example, Anisman et al., 2001; de Kloet and Molendijk, 2016; Shishkina et al., 2015). Thus, I considered that it would be an effective second hit factor in the context of the two-hit hypothesis.

2. Materials and methods

2.1. Animals

Eight nests of Wistar rats, each of which had a foster mother, and four male and three female pups, were raised in individual plastic cages. The pups in each nest were originally borne by two to four different mothers, and were randomly assigned to the nest after birth; however, all of them were born on the same day. Only male pups were subjected to drug treatment with MK-801 or saline (SAL). Thus, 32 pups received drug treatment and were prepared for behavioral testing. On postnatal days (PNDs) 7–20 (the day of birth was defined as PND 0), animals were injected subcutaneously with (+)-MK-801 hydrogen maleate (Sigma-Aldrich, St. Louis, Missouri, USA; 0.2 mg/kg) dissolved in SAL, or an equal volume of SAL (1 ml/kg) twice daily. Each animal was assigned to one of the two drug groups so that the mean pre-treatment body weight of the groups was almost equal. An interval of > 8 h was interposed between each drug treatment. The animals were weaned at PND 28, and thereafter housed individually in stainless steel wire cages with food and water *ad libitum*. Throughout the experimental period, subjects were maintained on a 12:12 h light–dark cycle. This study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and approved by the committee for animal research in Osaka City University.

2.2. Apparatus

For the prepulse inhibition test, an acoustic startle apparatus (BTI 1000, Bio-Medica Ltd., Osaka, Japan) was used. It contained an animal holder placed in a sound-attenuated chamber (52 cm wide × 38 cm long × 37 cm high inside dimensions) which had a ventilation fan and a small light bulb on an inner sidewall. The holder was made of semi-transparent polyvinyl chloride, and consisted of a cylinder (11 cm in diameter, 25 cm long) attached to a square platform (20 cm wide × 30 cm long). Each corner of the platform was unsteadily supported by a polyurethane ball (4 cm in diameter) placed on a short acrylic pipe (3.5 cm in diameter, 5 cm long). Vibrations derived from the rat's startle responses in the cylinder were detected by a piezoelectric acceleration sensor below the platform. The detected responses were acquired by a data recording system (PowerLab 2/26; ADInstruments, Bella Vista, New South Wales, Australia), and converted to numeric data by data-processing software (LabChart; ADInstruments) running on a Microsoft Windows-based computer. Three speakers that were used to produce white noise were horizontally placed on the ceiling of the chamber so that they were above the cylinder. Each of the speakers separately produced continuous background noise, and prepulse and pulse stimuli. Illumination inside the apparatus was approximately 20 lx. The volume of background noise, prepulses, and pulses was adjusted to 70, 75 and 105 dB, respectively.

A Ψ-shaped three-way maze made of gray polyvinyl chloride was used in the spontaneous alternation experiment. This maze consisted of a stem (12 cm wide × 50 cm long), three goal boxes (12 cm wide × 50 cm long for each), and a roughly hexagonal choice area (31 cm wide at its widest point, and 19 cm long at its longest point) between the stem and the three goal boxes. The closed end of the stem included a start box (12 cm wide × 15 cm long). The central goal box was directly connected with the stem *via* the choice area. Each of the left and right goal boxes extended from the root of the central goal box at an angle of approximately 45°. The central goal box was always closed by a guillotine door, and was not used in the experiment. The guillotine doors of the other goal boxes and that of the start box were always removed. The left and right goal boxes and the stem were used to assess the choice of the rat in the experiment. The illumination of the choice point was approximately 360 lx.

A gray square box (90 × 90 cm, 30 cm in height) was used as an open field. It was made of polyvinyl chloride, and had walls and a floor. The floor was divided into 25 (5 × 5) sections, each of which was 18 × 18 cm. A white bulb was suspended over the center of the apparatus, and the illumination of the center of the floor was approximately 300 lx.

A transparent acrylic cylinder pool (20 cm in diameter, 49 cm in depth) was used for the forced swim. It was filled with water (25 ± 1 °C), to a depth of 30 cm. The behavior of the rat was observed from the side of the pool *via* a web camera. The immobility of the rat in the pool was measured by video-tracking software (ANY-maze; Stoelting Co., Wood Dale, Illinois, USA) running on a Microsoft Windows-based computer. In order to obtain a good contrast between the background and the rat, several drops of black ink were put into the water.

2.3. Procedures

Each of the drug groups within each nest consisted of two rats, and they were subdivided into two subgroups; no stress or stress. Thus, four rats within each nest were assigned to one of four experimental groups: SAL–no stress, SAL–stress, MK-801–no stress, and MK-801–stress. Since one rat treated with MK-801 died during the drug treatment period, the number of rats in the MK-801–no stress group was seven, while there were eight rats in the other three groups.

Rats in the stress subgroups were subjected to 20 days of a forced-swim stress session (5 days/week × 4 weeks) from PND 56. During this period, rats were individually put into the water-filled forced-swim pool for 15 min daily.

From the day after the stress session had finished (PND 82), behavioral testing began. This consisted of prepulse inhibition (PPI), spontaneous alternation (SA), open-field, and forced-swim tests. These tests were conducted in the following order: PPI (days 1–3); SA and open field (day 4); forced swim (day 5).

2.3.1. PPI

The PPI test was conducted to measure sensorimotor gating in rats. On days 1 and 2, rats underwent a baseline session immediately