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

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Highlights

- Stress-vulnerability in rats neonatallty treated with MK-801 was examined.
- Rats were subjected to chronic forced-swim stress.
- Neonatal MK-801 treatment reduced stress-induced immobility in the forced-swim test.
- Neonatal MK-801 treatment may impair adaptation or coping ability against stress.
- Discussing the results in terms of the two-hit hypothesis of schizophrenia.

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.

Key words: NMDA receptors; Neonatal treatment; Animal model of schizophrenia; Prepulse inhibition; Stress coping; Working memory; Open-field; Rats

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.

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-piperadine carboxylic acid) and noncompetitive (MK-801) NMDA receptor antagonists impaired spatial working memory in the radial-arm maze or the delayed nonmatching-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 pretreatment body weight of the groups was almost equal. An interval of more than 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 semitransparent 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 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 degrees. 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 minutes 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 following habituation. In habituation, a rat was placed in the cylinder for 10 min with background noise on. The baseline

session was conducted to stabilize startle responses. This session had 40 pulse-alone trials, in each of which a 40-ms pulse stimulus was presented. An interval of 25 s on average (ranging from 15 to 35 s) separated each trial.

On day 3, each rat was subjected to 5 min habituation followed by a 10-trial baseline session. Immediately after this, each rat received a test session. In this session, 20 pulse-alone trials and 20 prepulse trials were given in a pseudorandom order with the exception being that fewer than five trials of the same type occurred consecutively. A prepulse trial had a 20-ms prepulse stimulus presented 100 ms before the beginning of a pulse stimulus. Pulse-alone trials were identical to those in the baseline session. An interval of 25 s on average (ranging from 15 to 35 s) was interposed between each trial. Continuous background noise was presented throughout all the sessions on days 1–3.

In all of the pulse-alone and prepulse trials of the test session, 100 startle amplitudes were sampled every 1 ms immediately after onset of the pulse stimulus, and the startle response within each trial was defined as a summation of these 100 sampling values.

A mean value for the startle response was calculated for the pulse-alone trials and for the prepulse trials for each rat. Based on these means, a PPI score was calculated using the following formula, as the percentage inhibition by the prepulse stimuli on startle responses evoked by the pulses: % PPI = [1 - (mean startle response in the prepulse trials) / (mean startle response in the pulse-alone trials)] × 100.

2.3.2. SA

The rat was placed on the choice area of the maze, and allowed to freely explore the maze for 10 min. The three arms of the maze were provisionally designated arms A,

B, and C.

When all paws of the rat entered an arm, this arm was recorded as being entered. An SA was counted if three consecutive arm entries were different from each other. Percentage SAs, an index of working memory, was calculated by the following formula: % SAs = [(the number of SAs) / (total entries - 2)] × 100. For instance, if the rat entered the arms as follows: A B C B C A C B A C A, the number of SAs was counted as five (that is, “ABC”,“BCA”,“ACB”,“CBA”,“BAC” in order), so percentage SAs was [5 / (11 - 2)] × 100 = 55.6 %. If a repeat entry to one arm (for example, reentry into arm A immediately after a choice to A) was observed, it was not considered a choice. An animal in the MK-801–stress group that had only three choices was excluded from the data analysis of % SAs.

2.3.3. Open field

Each subject was put into a corner of the apparatus, and was allowed to move freely. The numbers of traversed sections and rearings were counted for five min.

2.3.4. Forced swim

The forced-swim test was carried out to examine stress coping ability in rats. It was identical to the forced-swim stress procedure, except that only one trial was conducted. Since the first 10 min was used as the accommodation period, the immobility of each rat in the last five min was measured.

2.3.5. Statistical analysis

All of the measures were tested by two-way (neonatal treatment × stress) analyses

of variance (ANOVAs). Percentages of SAs were transformed by an arcsine transformation to modify heterogeneity of variance prior to the ANOVA. If an interaction was revealed by an ANOVA, simple main effects were examined. Additionally, in the PPI test, the difference in startle responses between trial patterns (pulse-only vs. prepulse) was verified by a paired *t*-test in each of the four groups.

3. Results

3.1. PPI

PPI scores are shown in Fig. 1. The mean startle response in pulse-alone (panel A) and prepulse (panel B) trials are shown in Fig. 2. For all of these measures no significant differences were seen between experimental groups, although significant inhibitory effects of prepulse stimuli on startle responses were observed in all four groups [SAL–no stress: $t(7) = 3.57$, $p < .01$; SAL–stress: $t(7) = 3.44$, $p < .05$; MK-801–no stress: $t(6) = 3.12$, $p < .05$; MK-801–stress: $t(7) = 5.31$, $p < .01$].

3.2. SA

The mean percentage of SAs (panel A), and the mean number of total choices (panel B) are shown in Fig. 3. No significant differences in % SAs were seen between groups. There was a trend toward a decrease in total choices following MK-801 treatment [$F(1, 27) = 3.25$, $p = .083$].

3.3. Open field

The mean values of the numbers of sections traversed (panel A) and rearings (panel B) are shown in Fig. 4. Chronic stress significantly increased rearing activity [$F(1, 27) = 4.48$, $p < .05$]. On the other hand, there was a trend toward a decrease in the number of rearings following MK-801 treatment [$F(1, 27) = 3.36$, $p = .078$]. No significant differences with respect to the number of sections traversed were revealed among the groups.

3.4. Forced swim

The mean immobility times in the last five min of the test are shown in Fig. 5. Neonatal MK-801 treatment significantly reduced immobility [$F(1, 27) = 9.93$, $p < .01$]. Conversely, immobility was significantly increased by stress [$F(1, 27) = 14.87$, $p < .01$]. Since the neonatal treatment × stress interaction was also significant [$F(1, 27) = 6.88$, $p < .05$], the simple main effects were tested. In SAL-treated rats, but not those treated with MK-801, chronic stress significantly increased immobility [$F(1, 27) = 21.74$, $p < .01$], while MK-801 treatment significantly inhibited the potentiating effect of chronic stress on immobility [$F(1, 27) = 17.26$, $p < .01$], although the drug had no effect in absence of this stress.

4. Discussion

The present study has demonstrated that repeated neonatal MK-801 treatment reduces immobility in the forced-swim test, and rearing activity in the open-field test. In particular, this treatment inhibited the potentiating effect of chronic stress on immobility

in the forced-swim test. These results suggest that chronic neonatal NMDA receptor antagonism persistently affects cognitive or behavioral functions, and influences the effects of stress on these functions. Since the four behavioral tests were carried out in the same order, the sequence of testing could have affected the results of this study. However, as the tests here are not considered to be especially stressful for rats—with exception of the forced-swim test that was conducted at the end of the testing—, such effects may be small even if this is the case.

The most important result in the present study is that MK-801-treated rats showed markedly less immobility in the forced-swim test than those treated with SAL, when repeated forced-swim stress had been previously applied. To the best of my knowledge, this is the first study showing that neonatal NMDA receptor antagonism reduces immobility in the forced-swim test. This result suggests that SAL-treated rats can acquire an optimal coping strategy to conserve energy but rats treated with MK-801 cannot. Thus, this finding strongly suggests that neonatal MK-801 treatment alters stress coping or adaptation abilities.

This result also suggests that MK-801-treated rats are sensitive or vulnerable to stress. Since stress is a major second hit factor, this is consistent with the two-hit hypothesis which assumes that genetic or early developmental first hit factors may induce vulnerability to later second hit factors in adolescence or adulthood (Bayer et al., 1999; Feigenson et al., 2014; Maynard et al., 2001). Consequently, neonatal NMDA receptor antagonism may act as a first hit factor, conferring vulnerability to second hit factors. According to the two-hit hypothesis, animal models where the subject has been exposed to both first and second hit factors may be of value. In this regard, a previous study has shown that neonatal MK-801 treatment produces more robust behavioral or

294 cognitive alterations when combined with isolation rearing stress (Lim et al., 2012). It
295 suggests that two-hit models have greater validity and application as animal models of
296 schizophrenia than those based on a first hit factor only, such as animals subjected to
297 neonatal MK-801 treatment alone.

298 Other possibilities apart from stress-coping or adaptation disabilities could explain
299 the decreased immobility in the forced-swim test seen in MK-801-treated rats. Firstly,
300 MK-801 treatment could increase general sensitivity to outer stimuli. This is supported
301 by the authors' observation that MK-801-treated rats were hypersensitive to external
302 stimuli, which typically manifested as jumping or squealing upon being touched
303 (Kawabe and Miyamoto, 2008). Secondly, neonatal MK-801 treatment could simply
304 increase locomotor activity. However, contrary to this hypothesis, this treatment did not
305 increase general activity, and even reduced the number of rearings, in the open-field test
306 of the present study. Thirdly, it is possible that MK-801-treated rats are less depressive,
307 since immobility in the forced-swim test is frequently used as an indicator of depression
308 or lower motivation. According to this view, reduced immobility in MK-801-treated rats
309 may reflect a higher resistance to stress. However, it appears that rodents in a forced-
310 swim situation show active coping styles in the earlier stage, for example, struggling,
311 jumping or swimming in the water; then switch to a passive coping strategy to conserve
312 energy, for example, immobile or floating behavior (de Kloet and Molendijk, 2016). It
313 seems, therefore, that passive coping styles are more adaptive than active ones. In
314 particular, as the rats in the chronic stress groups had fully experienced the forced-swim
315 situation, it is likely that the observed immobility reflects coping or adaptation ability
316 rather than depression or lower motivation. At any rate, further studies using a different
317 procedure are required to measure stress coping or depression in rats subjected to

318 neonatal NMDA receptor antagonism and chronic stress.

319 Alternatively, less immobility in MK-801-treated rats could be attributed to
320 memory problems. Since subjects had sufficient opportunity to acquire an optimal
321 coping strategy during repeated exposure to the forced-swim paradigm, it is possible
322 that MK-801-treated rats had memory deficits related to the stressful situation. This type
323 of memory includes episodic or contextual memory, for which normal hippocampal
324 function is needed (for example, Phillips and LeDoux, 1992). In fact, previous studies
325 have indicated that chronic neonatal treatment with NMDA receptor antagonists
326 produces memory deficits which may be attributed to hippocampal dysfunction (Gorter
327 and de Bruin, 1992; Kawabe et al., 2007; Kawabe and Miyamoto, 2008; Sircar, 2003).
328 However, memory problems alone cannot explain reduced immobility in MK-801-
329 treated rats, considering that these subjects tended to be less immobile even if they had
330 not experienced the stressful situation previously.

331 It has been reported that neurons in the hippocampus (Conrad et al., 1999;
332 Watanabe et al., 1992; Wood et al., 2004; Woolley et al., 1990) and the prefrontal
333 cortex (Hains et al., 2009; Radley et al., 2004) are degenerated by stress or
334 corticosterone, one of the principal adrenal glucocorticoids secreted by rodents in
335 response to stress. These areas are known to be rich in glucocorticoid receptors, and it is
336 believed that negative feedback is relayed from these regions to the hypothalamic–
337 pituitary–adrenal (HPA) axis *via* glucocorticoid receptors (Akana et al., 2001; Diorio et
338 al., 1993; Herman et al., 1989; Sapolsky et al., 1984). Since it has been suggested that
339 neonatal NMDA receptor blockade induces upregulation of NMDA receptors in the
340 hippocampus and the frontal cortex (Sircar, 2003), and that stress or corticosterone
341 treatment enhances glutamate release in these areas (Hascup et al., 2010; Lowy et al.,

342 1993; Moghaddam, 1993; Venero and Borrell, 1999), they may be more vulnerable to
343 excitotoxicity based on excessive glutamatergic transmission due to stress exposure in
344 MK-801-treated animals. Neural degeneration of these areas induced in this manner
345 could bring about disinhibition or hyperactivation of the HPA axis, which may then act
346 as a possible stress-vulnerability factor in these animals. In addition, the prefrontal
347 cortex is reciprocally connected with the limbic system including the hippocampus and
348 the amygdala, and generally thought to utilize information from these areas for
349 appropriate decision-making. Therefore, irregular stress coping in MK-801-treated rats
350 could be a consequence of abnormal decision-making based on prefrontal dysfunction.
351 In this regard, it has been reported that neonatal MK-801 treatment impairs cognitive
352 functions which are related to prefrontal performance such as cognitive flexibility
353 (Stefani and Moghaddam, 2005) and working memory (Kawabe et al., 2007; Kawabe
354 and Miyamoto, 2008; Stefani and Moghaddam, 2005).

355 In the open-field test, there was a trend toward decreased rearing activity following
356 MK-801 treatment, in accordance with previous studies (Kawabe et al., 2007; Uehara et
357 al., 2009). This may be related to the trend toward a decreased number of total choices
358 in the present SA test induced by MK-801, suggesting that MK-801 treatment reduces
359 locomotor activity. In addition, since illumination in the present open-field study was
360 brighter than that in a typical living environment, the testing situation could have been
361 more stressful or anxiety-inducing for the experimental subjects. Thus, lower activity in
362 MK-801-treated rats might reflect increased stress or anxiety. On the other hand, the
363 present study has also shown that chronic stress increases the number of rearings in the
364 open-field test. This result is similar to some previous reports showing that chronic
365 stress enhances locomotor activity (Grønli et al., 2005; Mineur et al., 2006), although

other studies have reported a reduction in activity (Conrad et al., 1999; Taliaz et al., 2011; Wood et al., 2004). Open-field activity may be interpreted ambiguously, possibly indicating excitation or sedation, anxiety, novelty seeking or exploration, and so on. Furthermore, many environmental factors, including the lighting, the size of the arena, and the familiarity or novelty of the testing set-up, may affect the results of an open-field test. It should also be noted that horizontal activity, or the number of traversed sections, was not affected in this study by either MK-801 treatment or chronic stress. Therefore, other behavioral tests measuring emotionality or anxiety will be necessary to elucidate the nature of the decreasing trend seen with MK-801 treatment and augmentative effect of stress on rearing activity in this study.

Since working memory is profoundly impaired in schizophrenic patients (Goldman-Rakic, 1994; Manoach, 2003), this is often examined in animal models of schizophrenia. Indeed, working memory deficits are shown in such models (Enomoto and Floresco, 2009; Kawabe et al., 2007; Kawabe and Miyamoto, 2008; Lipska et al., 2002; Stefani and Moghaddam, 2005), including animals that have received neonatal treatment with NMDA receptor antagonists. Our previous studies showed that the same dose (0.2 mg/kg) and schedule (PND 7–20, twice daily) of neonatal MK-801 treatment as in the present study impaired working memory in the radial-arm maze (Kawabe et al., 2007) and the DNMTP (Kawabe and Miyamoto, 2008) tasks. However, the present study failed to show that this MK-801 treatment protocol impaired working memory in the SA test, even if the rats had undergone a chronic stress procedure. This discrepancy may be caused by the task characteristics, or whether external rewards are needed or not. Since the SA test does not require external rewards, it is essentially different from reward-motivated tasks including the radial-arm maze and the DNMTP tasks.

Considering previous reports showing that animals that have received neonatal treatment with NMDA receptor antagonists are behaviorally sensitive to direct and indirect dopamine receptor agonists in adolescence or adulthood (Beninger et al., 2002; Dall'Olio et al., 1994; Uehara et al., 2010), neonatal NMDA receptor antagonism may induce hyperactivity of the dopaminergic system which is involved in reward-related behaviors. In this regard, it has been reported that neonatal NMDA receptor antagonism increases dopamine D_2 receptor binding in the nucleus accumbens, which is a major component of the reward system (Dall'Olio et al., 1994). More simply, it is also possible that the SA test is less sensitive to working memory deficits than the DNMTP and the radial-arm maze tasks. However, irrespective of the underlying reason for the differences, it would be worthwhile to assess the stress-vulnerability of MK-801-treated rats using an alternative working memory test.

As severe impairments in PPI are associated with schizophrenia, it is valuable to measure this parameter in animal models of the disorder. Although some studies show that neonatal NMDA receptor antagonism impairs PPI (Uehara et al., 2009; Uehara et al., 2010; Wang et al., 2003; Wedzony et al., 2008), the present study does not demonstrate such an effect in MK-801-treated rats, even after the application of chronic stress. However, since even SAL-treated rats showed comparatively low PPI values in this study, floor effects may have masked the impact of the drug and stress. In the PPI test, one of the critical factors is the difference between the sound intensity of prepulse stimuli and that of background noise. Related to this, many studies show that the higher the intensity difference, the greater PPI (for example, Beninger et al., 2002; Lim et al., 2012; Ralph et al., 1999). Thus, in the present study, a lower intensity difference may have contributed to the lack of PPI impairment by NMDA receptor antagonism and

414 stress. An experimental set-up using a higher prepulse intensity would be necessary to
415 achieve robust PPI values and better sensitivity to experimental effects.

416 In the present study, chronic stress loading started from PND 56, and behavioral
417 testing was conducted from PND 82. It is likely that the neonatal MK-801 treatment
418 was still effective in the testing period here, considering our previous studies show that
419 the same treatment severely impairs working memory in the radial-arm maze and the
420 DNMTP tasks at almost the same ages (11 weeks old and above) as those in the present
421 study (Kawabe et al., 2007; Kawabe and Miyamoto, 2008). However, unlike the
422 measures used in those previous studies, it may be the case that this treatment is no
423 longer effective with respect to the measures used in this particular study. In addition, it
424 is also possible that MK-801-treated rats have critical developmental periods where
425 stress or some second hit factors can affect these behavioral measures. Therefore,
426 application of stress and/or behavioral testing during younger periods, such as puberty
427 or adolescence, could reveal more profound effects of MK-801 treatment and/or stress.

428 In summary, the present study has shown that MK-801-treated rats may have a
429 reduced ability to habituate or adapt to a stressful context. Although the present study
430 supports the hypothesis that rats subjected to repeated neonatal treatment with NMDA
431 receptor antagonists are sensitive or vulnerable to stress, additional studies are required
432 to further test this hypothesis.

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685 Figure legends

686

687 Fig. 1. PPI scores (mean ± SEM).

688 Fig. 2. Startle response amplitudes in the pulse-alone trials (A) and the prepulse trials
689 (B) in the PPI test (mean ± SEM).

690 Fig. 3. Percentage spontaneous alternations (% SAs) (A) and the number of total
691 choices (B) in the spontaneous alternation test (mean ± SEM). † p <.1.

692 Fig. 4. The number of sections traversed (A) and the number of rearings (B) in the
693 open-field test (mean ± SEM). * p <.05, † p <.1.

694 Fig. 5. Immobility times in the last five min of the forced-swim test (mean ± SEM). ** p
695 <.01.

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698

Fig. 1

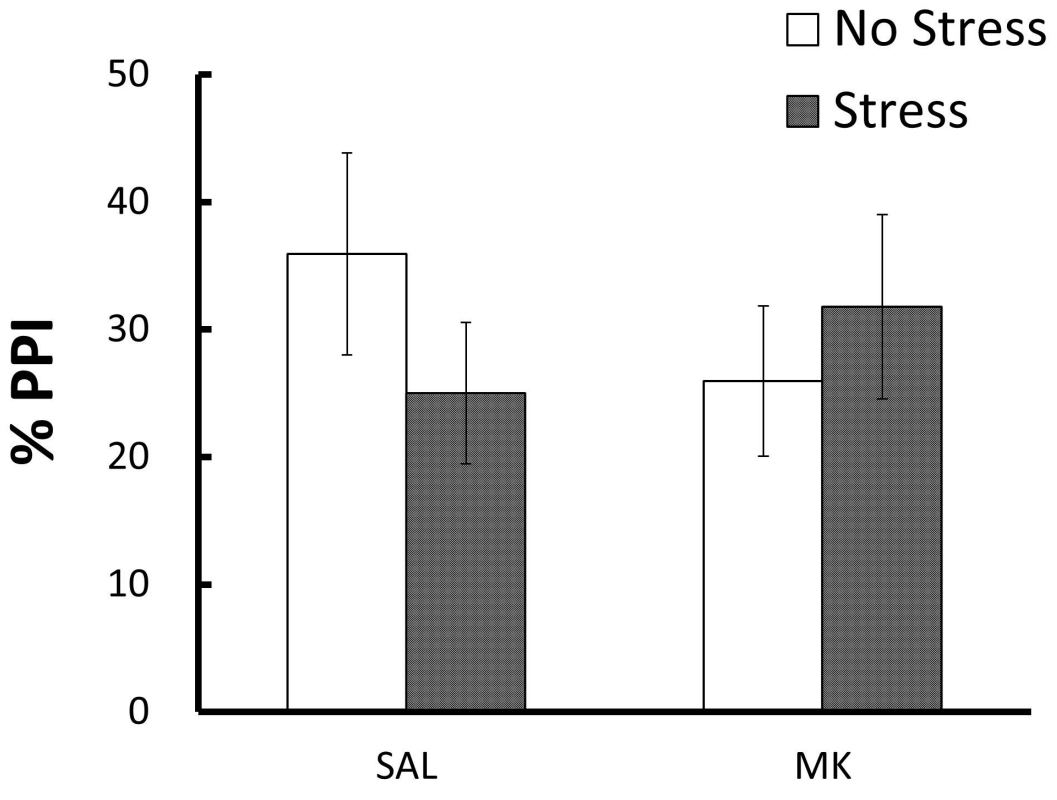
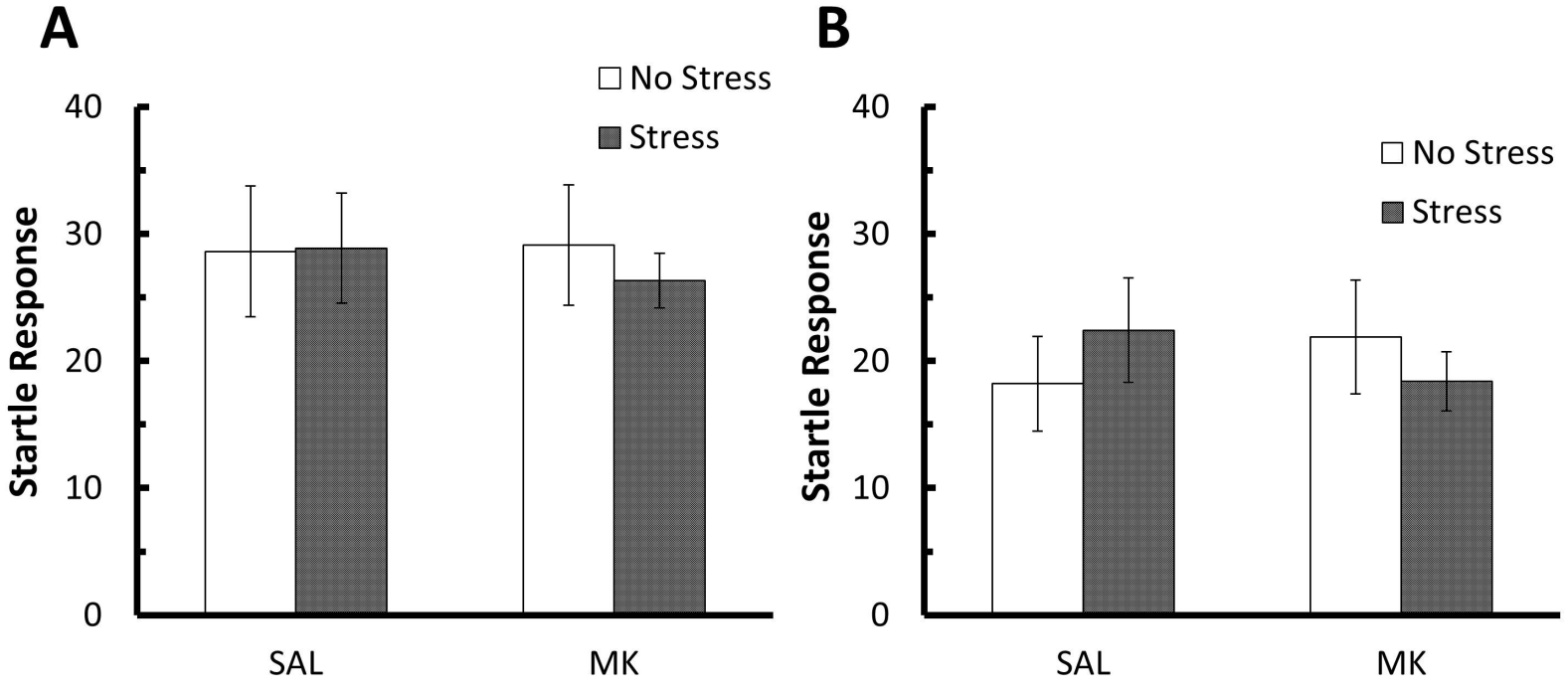


Fig. 2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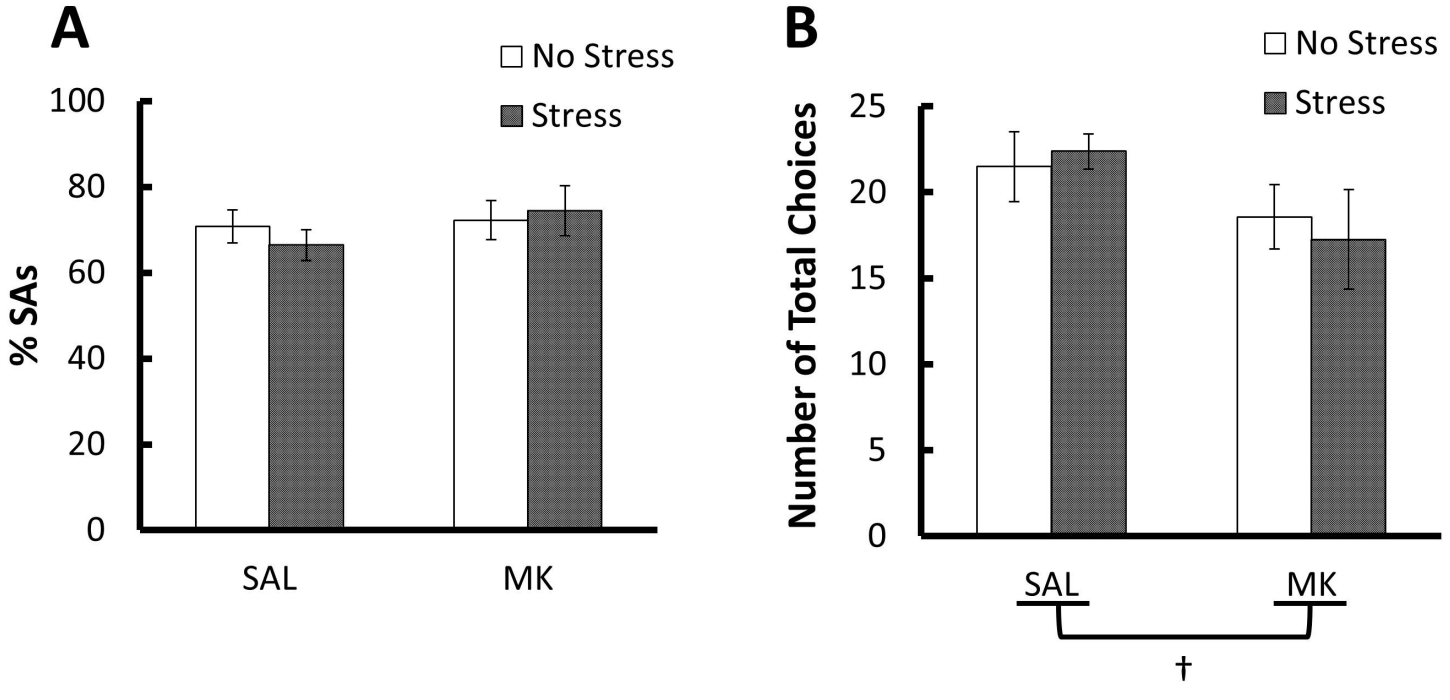
SAL
MK

Fig. 3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Stress
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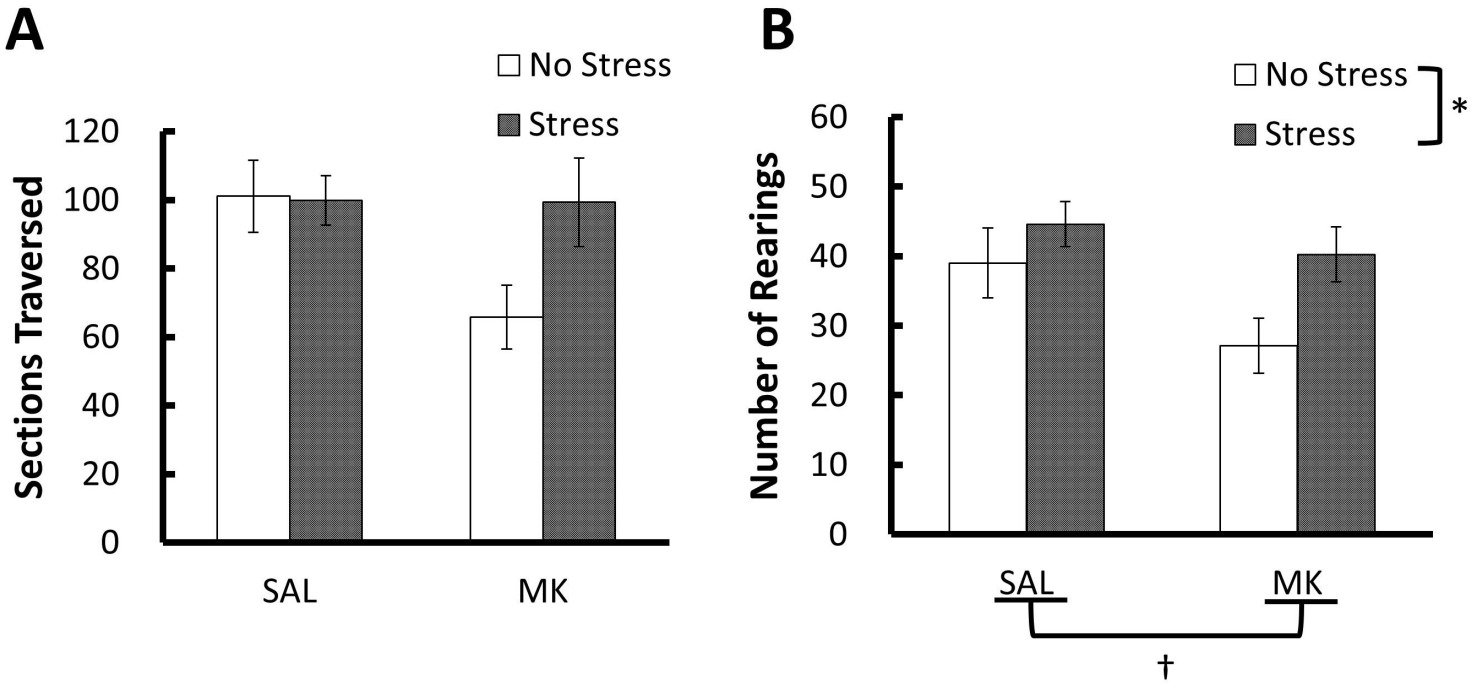
Fig. 4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Sections Traversed
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B
No Stress
Stress
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Number of Rearings
60
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SAL
MK
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Fig. 5