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Short communication

Chronic NMDA receptor blockade in early postnatal period, but not in adulthood, impairs methamphetamine-induced conditioned place preference in rats

Hiroki Furuie[a,b], Kazuo Yamada[a], Yukio Ichitani[a,*]

[a] Institute of Psychology and Behavioral Neuroscience, University of Tsukuba, Tsukuba, Ibaraki 305-8577, Japan
[b] Center for Medical Sciences, Ibaraki Prefectural University of Health Sciences, Ami, Ibaraki 300-0331, Japan

HIGHLIGHTS

- Rats with chronic NMDA receptor blockade were tested in conditioned place preference in adulthood.
- Early postnatal NMDA receptor blockade impaired conditioned place preference induced by methamphetamine.
- The same treatment in adulthood did not produce impairment.
- Abnormal development by NMDA receptor blockade may cause classical conditioning deficit.

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ABSTRACT

Early postnatal glutamatergic *N*-methyl-D-aspartate (NMDA) receptor blockade in animals is known to produce various behavioral deficits in adulthood. In the present study rats postnatally (day 7–20) treated chronically with MK-801, an NMDA receptor antagonist, were tested later in adulthood in methamphetamine (MAP)-induced conditioned place preference (CPP) using a unbiased procedure in a three-compartment apparatus. Rats with the same chronic treatment in adulthood were also tested. CPP test consisted of a baseline test before conditioning, place conditioning, and a preference test after conditioning. Rats postnatally treated with MK-801 did not show any evidence of preference for MAP-paired compartment compared with that for unpaired one in the preference test that was shown in rats postnatally treated with saline. On the other hand, rats treated with MK-801 in adulthood were not affected by the treatment and showed significant CPP as was shown in saline-treated control animals. Results suggest the possibility that chronic early postnatal, but not adulthood, NMDA receptor blockade induces persistent deficit of subsequent appetitive classical conditioning.

N-Methyl-D-aspartate (NMDA) receptors, an ionotropic glutamate receptor subtype, are known to play critical roles in learning and memory. It has been reported that NMDA receptor antagonists cause deficits in various types of learning and memory tasks [1–4]. In addition, NMDA receptors play important roles in early brain development. For instance, NMDA receptors mediate neuronal maturation, proliferation and migration in fetal or postnatal animals' brain [5,6]. Furthermore, NMDA receptors regulate neuronal cell survival during postnatal period, and NMDA antagonists such as phencyclidine (PCP), ketamine, and MK-801 (dizocilpine; [5R,10S]-[+]-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine), induce apoptotic neurodegeneration in rats [7]. Moreover, rats postnatally treated with NMDA receptor antagonists showed neuroanatomical, neurochemical and morphological abnormalities in the brain even long after the drug treatment [8,9]. Thus, NMDA receptor blockade in early postnatal period results in abnormal brain development, and these adverse effects on the brain are not transient but long-lasting, perhaps, throughout the life.

The alterations in the brain induced by postnatal NMDA receptor blockade are thought to affect many aspects of animals' behavior. In fact, in previous studies, animals postnatally treated with NMDA antagonists showed abnormalities in various behavioral tests in adulthood. For example, rats postnatally treated with NMDA receptor antagonists exhibited increased locomotor activ-

* Corresponding author at: Institute of Psychology, University of Tsukuba, Tennoudai 1-1-1, Tsukuba, Ibaraki 305-8577, Japan. Fax: +81 29 853 6094.
E-mail address: y-ichit@human.tsukuba.ac.jp (Y. Ichitani).

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ity, deficits in sensorimotor gating and attention, and impaired learning and memory especially in tasks which depend on the hippocampus such as water maze, radial-arm maze, and delayed nonmatching-to-position tasks [10–16]. Thus, the deficits in learning and memory observed in rats postnatally treated with NMDA antagonists are widely accepted. However, how postnatal NMDA receptor blockade affect classical conditioning has not been thoroughly investigated. Since classical conditioning is an important factor underlying animal learning and behavior, examining how postnatal NMDA receptor blockade may affect classical conditioning could elucidate changes in cognition and learning behavior in adulthood in general. Recently, we reported that rats postnatally treated chronically with MK-801 could acquire aversive classical conditioning [17]. We found that although MK-801 treatment in early postnatal period attenuated the inhibitory effect of conditioned stimulus (sucrose) pre-exposure on conditioned taste aversion (CTA), the treatment did not affect CTA conditioning itself or conditioned emotional response (CER). However, whether rats can also normally acquire appetitive classical conditioning still remains unclear.

Here, we addressed the question of whether chronic NMDA receptor blockade during the early postnatal period affects appetitive classical conditioning in later life by using conditioned place preference (CPP) test. To achieve this goal, we treated rats with MK-801 on postnatal days 7 through 20, and conducted methamphetamine (MAP)-induced CPP test in adulthood. In addition, we tested MAP-induced CPP in rats that received chronic MK-801 treatment in adulthood.

In order to assess the effects of chronic postnatal MK-801 treatment, 17 male rat pups obtained from six female Wistar-Imamichi rats (Institute for Animal Reproduction, Japan) were used. The day of birth was defined as postnatal day (PND) 0. To avoid malnutrition, the number of pups in each litter was culled to less than 10 on PND 6. Male rat pups received injections of 0.4 mg/kg of (+)-MK-801 hydrogen maleate (MK-801; Sigma, MO, USA) dissolved in physiological saline (SAL) or SAL (MK-801: $n = 8$; SAL: $n = 9$) subcutaneously twice per day on PND 7 through 20 (14 days). Each injection was spaced from the previous by more than 8 h. The dose and injection procedure of MK-801 was determined from previous studies [15–17]. Pups were weaned on PND 28 and thereafter they were housed three or four per cage.

In order to assess the effects of chronic MK-801 treatment in adulthood, 19 naive male Wistar-Imamichi rats (MK-801: $n = 9$; SAL: $n = 10$) were used. Chronic MK-801 treatment started at the age of 10 weeks. Rats were randomly assigned to two groups and received injections of 0.4 mg/kg of MK-801 or SAL in a similar manner to the postnatal treatment, described above, for 14 days. Rats were housed three or four per cage in a temperature-controlled room with a 12-h light–dark cycle (lights on 8:00–20:00) and allowed access to food and water ad libitum. In both postnatal and adult chronic treatments MK-801 produced a mild and transient ataxia immediately after each injection, however it disappeared soon after completion of drug treatment period. All behavioral testing was conducted during the light phase of light–dark cycle. Animal experiments were approved by the University of Tsukuba Committee on Animal Research.

For behavioral testing, two, three-compartment place preference chambers (MED Associates, USA) made of polyvinyl chloride were used. Each chamber consisted of a central small (neutral) compartment ($21 \times 12 \times 27$ cm) and two equally sized large (conditioning) compartments ($21 \times 27 \times 27$ cm) that were located on both sides of the neutral compartment. The neutral compartment had gray walls and its floor was made of gray polyvinyl chloride. One of two large compartments had white walls and its floor consisted of a metal grid, while the other compartment had black walls and its floor consisted of stainless steel rods. Each compartment was covered by transparent polyvinyl chloride lids and was separated from the other by a guillotine door (9×10 cm), and each door was operated by the experimenter. The apparatus was illuminated by ceiling lights. The illumination of the floor of the neutral compartment was 350 lx. Above the chambers, a video camera was suspended for recording the behavior of rats during the test.

Behavioral testing started at 16 weeks for rats with chronic treatment in postnatal periods, and at 14 weeks for rats with chronic treatment in adulthood. Handling and habituation to the apparatus preceded the conditioning. Rats received 5 min handling for 3 days and 10 min daily habituation to the apparatus for 3 days. During the habituation, rats were individually placed into the neutral compartment of the conditioning chamber, the guillotine doors were then opened and they were allowed to explore freely. After the habituation period, a baseline test was conducted and preference between two compartments was assessed. The rats were exposed to the chamber for 15 min in a similar way to the habituation. The time spent in each compartment was recorded and the preference score was calculated by dividing the time spent in each compartment by the total time spent in both compartments. Based on this preference score, which side of the two compartments (black or white) would be paired with MAP in the subsequent conditioning sessions for each rat was determined. This set the baseline preference for the compartment in each chronic treatment group to approximately 50% (unbiased procedure). Conditioning sessions started the day following the baseline test. Place preference conditioning was conducted for 4 days. Rats received a pairing of either drug or SAL with a specific compartment once a day, and pairings of MAP and SAL were alternately carried out 2 times each. During the conditioning session, rats were injected with 1.0 mg/ml/kg MAP (Sumitomo Dainippon Pharma, Japan) or SAL intraperitoneally, and immediately after the drug injection, they were confined to the predetermined compartment for 30 min. This dose was chosen since a significant rewarding effect of MAP on CPP was shown previously [18]. On the day following the last conditioning session, the preference test (15 min) was conducted in the same manner as the baseline test. Analysis of time spent in each compartment and the number of crossings was performed from video recordings by an experimenter blind to treatment groups (MK-801 or SAL) and which compartment the animal was paired with MAP in the conditioning session.

Mean preference scores were compared to the theoretical chance level (50%) using one-sample t-test. The preference score and the number of crossings between compartments in the baseline and preference tests were analyzed by two-way analysis of variance (ANOVA) for repeated measures followed by Bonferroni's post-hoc test. p Values less than 0.05 were considered to show statistically significant difference.

Fig. 1A shows the preference scores in the baseline and preference tests of rats that received chronic treatment in postnatal period. The preference scores of both treatment groups in the baseline test were not significantly different from chance levels. In the preference test, the score of postnatally SAL-treated rats was significantly higher than chance levels ($p < 0.01$), however the score of postnatally MK-801-treated rats was not. ANOVA (Chronic treatment × Phase: baseline vs. preference test) showed a significant main effect of Phase ($F(1,15) = 10.46$, $p < 0.01$). Interaction of Treatment × Phase was also significant ($F(1,15) = 6.79$, $p < 0.05$). Post-hoc tests revealed that in rats postnatally treated with SAL, but not in rats postnatally treated with MK-801, the preference score in the preference test was significantly higher than in the baseline test ($p < 0.01$). Fig. 1B shows the preference scores in the baseline and preference tests of rats that received chronic treatment in adulthood. The preference scores of both treatment groups in the baseline test were not significantly different from chance levels. However, the scores in the preference test of both groups