Neuroscience Letters 641 (2017) 33–39

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Long-term effects of maternal separation coupled with social isolation on reward seeking and changes in dopamine D1 receptor expression in the nucleus accumbens via DNA methylation in mice

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HIGHLIGHTS

- Early life stress by MS reduced reward seeking in adult female mice.
- The expression of dopamine D1 receptor in the NAc was decreased in adult MS females.
- The promoter region of the D1 receptor was hypermethylated by MS experience in female mice.

ARTICLE INFO

Article history:
Received 30 September 2016
Received in revised form
26 December 2016
Accepted 11 January 2017
Available online 19 January 2017

Keywords:
Conditioned place preference
Dopamine receptor
Early-life stress
Epigenetics
Reward

ABSTRACT

Early-life stress has long-lasting effects on the stress response, emotions, and behavior throughout an individual's life. Clinical reports have demonstrated that child abuse victims exhibit impairments in reward-associated behavior; yet, the mechanism for this effect remains unclear. Maternal separation (MS) or MS coupled with social isolation (SI) (MS + SI) is widely used as a model for early-life stress in rodent studies. We employed mice subjected to MS + SI to clarify the long-term effect of early-life stress on reward-seeking involving palatable foods by a conditioned place-preference (CPP) paradigm. Prior MS + SI experience decreased exploration time in a chocolate-paired compartment in adult female mice, but not in male mice. We then focused on the mesolimbic dopamine pathway associated with reward-seeking behavior and measured both mRNA and protein levels of tyrosine hydroxylase (TH) in the ventral tegmental area (VTA) and dopamine D1 and D2 receptors in the nucleus accumbens (NAc). MS + SI female mice had significantly lower D1 receptor mRNA and protein levels than controls, whereas the expression of TH and the D2 receptor was similar in the 2 groups. All mRNA and protein levels were unchanged in MS + SI male mice. When attempting to elucidate the mechanism underlying downregulation of the D1 receptor in the NAc of MS + SI females, we found hypermethylation of the *Drd1a* promoter region. These results suggest that early-life stress affects reward-seeking behavior in female mice, which may be associated with the downregulation of D1 receptor in the NAc via epigenetic modification of its promoter region.

1. Introduction

Early-life stress has long-lasting effects on an individual's stress response, emotions, and behavior throughout life, and increases the risk for various psychiatric diseases, including depression and posttraumatic stress disorder [1]. Recently, clinical report have indicated that child abuse victims exhibit reduced sensitivity to reward and show decreased activation of the striatum and thalamus during reward processing [2]. Maternal separation (MS) or

Abbreviations: CpG, cytosine-phospho-guanine dinucleotide; CPP, conditioned place preference; DNMT, DNA methyltransferase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MS, maternal separation; MS + SI, maternal separation coupled with social isolation; NAc, nucleus accumbens; PND, postnatal day; SI, social isolation; TH, tyrosine hydroxylase; VTA, ventral tegmental area.

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http://dx.doi.org/10.1016/j.neulet.2017.01.025
0304-3940/

MS coupled with social isolation (SI) (MS + SI) is an animal model for studying the mechanisms underlying the effects of early-life stress on the development of psychiatric disorders. Animal studies related to early-life stress have revealed that an abnormal maternal environment, including MS, can impair innate reward-seeking behavior [3–5]. The mesolimbic dopamine system is a key brain circuit to regulate reward seeking, which comprises dopaminergic neurons that project from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) [6]. The principal cells of the NAc are medium spiny neurons that express $G_{s\alpha}$-coupled dopamine D1 receptors and $G_{i\alpha}$-coupled dopamine D2 receptors. Although some reports have demonstrated that early-life stress decreased dopamine receptor expression [7,8], the molecular mechanisms underlying receptor regulation remain unclear.

Epigenetic modifications, such as DNA methylation of promoters and histone modification regulate gene expression and have been implicated in the pathologies of several psychological disorders [9]. DNA methylation involves methyltransferase-catalyzed direct covalent addition of a methyl group to the fifth position of cytosine residues within cytosine-phospho-guanine dinucleotide (CpG); at gene promoters, this typically represses gene transcription. Importantly, MS and MS + SI is known to increase the expression of DNA methyltransferases (DNMTs) in the NAc of infant and adult rats [10–12]. Furthermore, different rearing environments during the first postnatal week in rodents modify epigenetic regulation of the glucocorticoid receptor gene [13]. Thus, MS + SI is likely to alter expression of several genes at epigenome level.

In this study, we hypothesized that early-life stress has long-lasting effects on reward-seeking behavior by altering the mesolimbic reward system. We assessed this using the MS + SI paradigm and examined reward-seeking behavior by a conditioned place preference (CPP) test, using chocolate as reward. To clarify whether MS + SI affects the mesolimbic dopamine system, we examined both mRNA and protein levels of tyrosine hydroxylase (TH) in the VTA and dopamine D1 and D2 receptors in the NAc. Furthermore, we evaluated DNA methylation of the D1 receptor promoter region in the NAc to elucidate the mechanism underlying expression of abnormal reward-seeking behavior induced by MS + SI.

2. Materials and methods

2.1. Animals

The numbers of animals used for each experiment are shown in Fig. 1. C57BL/6N female mice at day 14 of pregnancy were purchased from Japan SLC Inc. (Hamamatsu, Japan). Pregnant mice were randomly assigned to control (n = 18) or MS + SI (n = 18) groups and individually housed on a 12-h light/dark cycle (lights on at 08.00 h), in a temperature-controlled facility (23 °C) with 55% relative humidity. Standard laboratory chow and water were given *ad libitum*. The date of birth was designated as postnatal day (PND) 0. All animal protocols were approved by the Animal Care Committee of Nara Medical University and were performed in accordance with the policies established in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Maternal separation coupled with social isolation

The experimental schedule of MS + SI is shown in Fig. 1A and the procedure was performed as described previously [14]. In brief, pups in the MS + SI group were subjected to MS + IS for 3 h (09.30 – 12.30) daily from PND 1–14. During MS + SI sessions, each pup was isolated in a separate cup on a heating pad maintained at 32 °C, separated from the dams. Pups in the control group were left undisturbed with the dam until weaning, except for cage-cleaning once a week. All pups were weaned on PND 21 and housed in same-sex groups of 3 – 5 mice. After weaning, body weight and food consumption were measured at the time of weekly cage-cleaning.

2.3. CPP test

The CPP schedule including the pre-test, conditioning phase, and test and the numbers of animals used are shown in Fig. 1B. The CPP test was performed during the light phase (13.00 – 18.00) with mice (3 – 4 months of age) in a place-conditioning apparatus that consisted of 2 equal-sized compartments (20 × 20 × 25 cm) separated by a sliding door (O'Hara & Co., Ltd, Tokyo, Japan). The compartments had differently colored walls (black or white) and distinct floor textures (bar or grid). Mice were transferred to this room 1 h prior to the start of testing each day. CPP comprised 3 phases on consecutive days: pre-conditioning (2 days), conditioning (12 days), and testing (1 day). During the pre-conditioning phase, the sliding door was open and mice were allowed free access to both compartments for 15 min to determine the baseline box-preference of each mouse. Mice showing a biased baseline preference >80% or a difference in length of stay > 200 s during the pre-conditioning phase were omitted. During the conditioning phase, mice were confined to 1 compartment per day, with compartments alternated daily, by closing the sliding door for 30 min. Confinement to the preferred box was accompanied by access to standard laboratory chow, while confinement to the non-preferred box was accompanied by access to milk chocolate (Meiji Co., Ltd., Tokyo, Japan). On the testing day, the sliding door was opened, and mice were again allowed free access to both compartments for 15 min. Preference scores were expressed as the change in the time (s) spent in the chocolate-paired compartment before and after conditioning.

2.4. Food-choice test

A food-choice test was performed to evaluate the preference for chocolate under conditions in which mice were not required to seek reward (chocolate), by presenting chocolate and standard laboratory chow simultaneously to mice. The test was performed for 6 days continuously (see Fig. 1C), using 3 – 4-month-old female mice naïve to chocolate. Mice were individually transferred to a new cage daily, and given milk chocolate and standard laboratory chow simultaneously for 30 min. Mice were then returned to their home cage and the amounts of the foods consumed were measured. Food-choice ratios were calculated as the amount of chocolate consumed relative to the total amount of food consumed.

2.5. Tissue collection

Mice (11-week-old) were anesthetized and decapitated, and the brains were harvested for real-time polymerase chain reaction (PCR), western blot, and DNA methylation analysis. Brain slices were cut using a linear slicer (Pro.7; DOSAKA EM Co., Ltd., Kyoto, Japan) based on a mouse brain atlas [15], and the 2 slices that included the VTA (Fig. S1A-B, from bregma −3.16 mm, 350-μm thickness) and NAc (Fig. S1C-D, from bregma +1.70 mm, 600-μm thickness) were mounted on a rubber pad. The bilateral VTA was collected using a 21-gauge needle with a rounded tip. The bilateral NAc was collected using a puncher with 1.5-mm diameter after removing the anterior commissure using a 23-gauge needle with a rounded tip. Collected samples were stored at −80 °C until use.