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Research report

Hyperlocomotor activity and stress vulnerability during adulthood induced by social isolation after early weaning are prevented by voluntary running exercise before normal weaning period

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HIGHLIGHTS

- Social isolation after early weaning (EI) induces vulnerability to stress.
- EI increases locomotor activity in a novel environment.
- Exercise during juvenile prevents some of the behavioral abnormalities induced by EI.

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ABSTRACT

In rodents, the disruption of social-rearing conditions before normal weaning induces emotional behavioral abnormalities, such as anxiety, motor activity dysregulation, and stress vulnerability. The beneficial effects of exercise after normal weaning on emotional regulation have been well documented. However, effects of exercise before normal weaning on emotion have not been reported. We examined whether voluntary wheel running (R) during social isolation after early weaning (early weaning/isolation; EI) from postnatal day (PD) 14–30 could prevent EI-induced emotional behavioral abnormalities in Sprague–Dawley rats. Compared with control rats reared with their dam and siblings until PD30, rats performed R during EI (EI + R) and EI rats demonstrated greater locomotion and lower grooming activity in the open-field test (OFT) during the juvenile period. Juvenile EI ± R rats showed greater learned helplessness (LH) after exposure to inescapable stress (IS; electric foot shock) than IS-exposed control and EI rats. In contrast, EI rats showed increased locomotion in the OFT and LH after exposure to IS compared with control rats during adulthood; this was not observed in EI ± R rats. Both EI and EI ± R rats exhibited greater rearing activity in the OFT than controls during adulthood. EI did not increase anxiety in the OFT and elevated plus-maze. These results suggested that R during EI until normal weaning prevented some of the EI-induced behavioral abnormalities, including hyperlocomotor activity and greater LH, during adulthood but not in the juvenile period.

1. Introduction

Early social environments have long-lasting influences on emotional regulation of animals, including humans. Manipulations of social-rearing conditions before normal weaning, such as maternal deprivation, early weaning, and social isolation after early weaning (early weaning/isolation; EI), are widely used as disruption models of early social environments in rodents. These animal models have suggested an association between disrupted early social environments and symptoms of neuropsychiatric disorders, including anxiety, motor activity dysregulation, and high stress vulnerability. For example, repeated maternal deprivation during the neonatal period induces hyperlocomotor activity in novel environments,

Abbreviations: ACTH, adrenocorticotropic hormone; ADHD, attention-deficit/hyperactivity disorder; DA, dopamine; EI, early weaning/isolation; EPM, elevated plus-maze; G, group-reared after PD 30; HPA, hypothalamic pituitary-adrena; I, isolated; IS, inescapable stress; LH, learned helplessness; NA, noradrenaline; NS, non-stressed; OFT, open-field test; PD, postnatal day; R, running; TD, test day; 5-HT, serotonin.

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increases anxiety and depressive behaviors [1–5], and enhances stress vulnerability in rats [6]. EI induces hyperlocomotor activity in novel environments and anxiety-like behaviors in rats [7] and [8]. However, rats reared with their siblings after early weaning did not induce hyperlocomotor activity in a novel environment [9], but causes increased anxiety behaviors in rats and mice [9–11]. The harmful effects of disrupted early social environments have been well documented. However, early environments that could prevent the development of these harmful effects are not well known.

Voluntary exercise after early weaning has beneficial effects on emotional regulation. For instance, four weeks of voluntary wheel running (R) elicit typical anxiolytic responses in the elevated plus-maze (EPM) test in mice [12]. Furthermore, 6 weeks of R reportedly blocks the development of learned helplessness (LH), an animal model of depression, induced by inescapable stress (IS) in rats [13,14]. Similarly, 3 weeks of R reduces the locomotor activity of spontaneously hypertensive rats, which are widely used as an animal model of attention-deficit hyperactivity disorder (ADHD). However, the number of reports on the effects of exercise on developing animals is limited. Maniam and Morris [15] have reported that the initiation of voluntary running exercise immediately after normal weaning on postnatal day (PD) 20 attenuates anxiety- and depression-like behaviors that are induced by neonatal repeated maternal deprivation in rats [15]. To the best of our knowledge, effects of exercise before normal weaning on subsequent emotional regulation have not yet been reported.

In our previous study, we found that EI impairs monoaminergic axonal development, and these alterations are prevented by the initiation of R, immediately after initaiting EI at PD14 until PD28 [16]. Monoamines play important roles in brain development [17–20], and the impairment of monoaminergic axons during the juvenile period causes emotional abnormalities [21–24]. These findings raise the hypothesis that R during EI from PD14 to PD28 could prevent the development of EI-induced emotional behavioral abnormalities. To verify the validity of this hypothesis, male Sprague–Dawley rats were weaned at PD14 and separated from their dam and siblings until PD30, and their emotional behaviors were compared with EI animals and comparable animals that were allowed to perform R. Open-field tests (OFTs) were performed to investigate anxiety, locomotion, grooming, and rearing activity. EPM tests were performed to investigate anxiety. IS associated LH development was analyzed to estimate vulnerability to stress.

2. Methods

2.1. Animals

All Sprague–Dawley rats were housed under constant conditions (22 °C, humidity of 60 ± 5%, and 12-h light/dark cycle) with ad libitum access to food (MF, Oriental Yeast, Japan) and water. Each pregnant rat was placed in a separate plastic home cage (length, 40 cm; width, 25 cm; and height, 25 cm) with woodchip bedding (Charles river, Japan). Female rats (aged 8–11 weeks) were housed overnight with adult male rats to allow mating; the following morning, vaginal smears were examined. The sperm-positive day was designated embryonic day 0. All efforts were made to minimize the number of animals used and their suffering. The experimental conditions and procedures were approved by the Committee of the Ethics on Animal Experiments at Yamaguchi University Graduate School of Medicine. All manipulations and protocols were performed according to the Guidelines for Animal Experiments at Yamaguchi University Graduate School of Medicine and in accordance with the Japanese Federal Law (no. 105), Notification (no. 6) of the Japanese Government, and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publications no. 80-23), which was revised in 1996.

2.2. Experimental model

A previously described weaning procedure was used in this experiment [16]. Male pups were weaned and isolated on PD14 and individually housed until PD30 in plastic cages with running wheels (Melquest, Toyama, Japan). However, the running wheels were locked, so that they were incapable of rotating, for the EI animals, and were unlocked for the EI + R animals. To measure the running distance of the EI + R animals, the running wheels were equipped with a revolution counter (OMRON, Kyoto, Japan). The wheel revolution counts were noted every alternate day, and the running distance was calculated as the wheel circumference (1.0 m) × the number of revolutions. Some running wheels were a kind gift from Dr. Hitoshi Hirano (Health Administration Center, Yamaguchi University). Body weights (g) of rats were measured every alternate day. After early weaning, EI and EI + R rats were fed an ordinary adult diet comprising protein, lipids, carbohydrates, vitamins, minerals, and amino acids, and which was softened by soaking in water. The maternal care levels are different for each dam, and the mental development of the pups is extremely affected by the maternal care levels during the neonatal period. To eliminate the differences in mental development caused by differences in maternal care levels by each dam, pups from one mother were divided into three groups: the control, EI, and EI + R. Furthermore, the average body weights of three groups at PD14 were made to be the same. The control pups were housed in a cage with their dam and two or three pups until PD30. All female pups were removed from their home cages at PD8. At PD30, the control pups were weaned and housed in individual cages, and the running wheels were removed from the cages of the EI and EI + R groups.

2.2.1. Measurement of spontaneous motor activity

Spontaneous motor activity in 24 h and in the light and dark phases was measured in the home cages of rats with the SUPER-MEX activity-monitoring system (Muromachi Kikai, Tokyo, Japan), as previously reported [25] and [26]. The spontaneous motor activity of juvenile rats (Control: $n = 16$, EI: $n = 13$, and EI + R: $n = 13$) and adult rats (Control: $n = 9$, EI: $n = 8$, and EI + R: $n = 8$) were measured at PD30–PD35 and postnatal week 9, respectively.

2.3. Behavioral test procedures

The timeline of the testing procedure is shown in Fig. 1. In this study, animals were separated into 18 lines. To investigate behavioral responses of the first time in each test, the animal lines that were used for the juvenile behavioral tests were separated from those used for the adult behavioral tests. Emotional behaviors may change with each passing day during the juvenile period because the rat brain is developing at a remarkable pace during this period. To perform behavioral tests within almost the same developmental stage of the juvenile period, the animal lines used for the OFT and EPM tests were separated from those used for the LH test. Because the period between PD14 and PD28 is indicated to be long enough to exert beneficial effects of R [16], OFT, and IS, the first process of the LH test, were performed at PD28. Furthermore, animal lines for the OFT/EPM and LH tests during the adult period were separated in the same way as those for juvenile tests.

2.3.1. OFT

For the OFT, 28-day- (Control: $n = 18$, EI: $n = 8$, and EI + R: $n = 13$) and 9-week-old (Control: $n = 9$, EI: $n = 8$, and EI + R: $n = 8$) rats were used. The open field was a circular surface with a 60-cm diameter that was divided into 19 zones by lines. The field was enclosed by