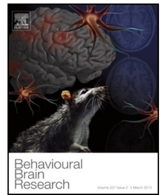
Behavioural Brain Research 284 (2015) 103–111

Contents lists available at ScienceDirect

Behavioural Brain Research

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



Research report

Anxiolytic-like effects of restraint during the dark cycle in adolescent mice

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HIGHLIGHTS

- Restraint during dark cycle reduced anxiety-like behaviors in adolescent mice.
- Restraint during light cycle increased anxiety behaviors in adolescent mice.
- Repeated dark cycle restraint for 2 weeks had a similar anxiolytic effect.
- In contrast, restraint during the light cycle produced anxiety behavior.
- Light cycle restraint altered the corticosterone levels and brain serotonin turnover.

ARTICLE INFO

Article history:
Received 19 December 2014
Received in revised form 1 February 2015
Accepted 5 February 2015
Available online 14 February 2015

Keywords:
Restraint
Anxiety behavior
Dark cycle
Light cycle

ABSTRACT

Stress during developmental stage may cause psychological morbidities, and then the studies on stress are important in adolescent rodents. Restraint is used as a common stressor in rodents and the effects of restraint during the light cycle have been studied, but those of restraint during the dark cycle have not. The present study examined the effects of restraint during the light and dark cycles on anxiety behaviors in adolescent mice. Restraint for 3 h during either the light or dark cycle impaired memory function in the fear conditioning test, but did not affect locomotor activity. In the elevated plus-maze test, restraint during the dark cycle reduced anxiety-like behaviors in mice. Repeated exposure to a 3-h period dark cycle restraint for 2 weeks had a similar anxiolytic-like effect. In contrast, restraint for 3 h during the light cycle produced anxiety behavior in adolescent, but not adult, mice. The light cycle stress increased plasma corticosterone levels, and elevated c-Fos expression in the prefrontal cortex, paraventricular hypothalamic nucleus, basolateral amygdala and dentate gyrus, and enhanced serotonin turnover in the hippocampus and striatum, while the dark cycle stress did not. There was no difference in the stress-mediated reduction in pentobarbital-induced sleeping time between dark and light cycle restraint. These findings suggest that the anxiolytic effect of dark cycle restraint is mediated by corticosterone, serotonin or γ-aminobutyric acid-independent mechanisms, although the anxiogenic effect of light cycle restraint is associated with changes in plasma corticosterone levels and serotonin turnover in specific brain regions.

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1. Introduction

Environmental factors play a key role in the development of brain functions [1–3]. Previous studies show a link between exposure to stress and the perturbation of many neurobehavioral processes [4,5]. In most previous studies, rodents were exposed to a particular condition continuously. But, it appears that the effects

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http://dx.doi.org/10.1016/j.bbr.2015.02.010
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of environmental stress depend on the time and duration of exposure to the stress. We recently found that social crowding during the dark, but not light, cycle produces anxiolytic-like effects in adolescent mice [6], suggesting that the effect of stress is dependent on circadian cycle exposed to stress.

Restraint has been extensively used to study the role of stress in psychopathology. Previous studies have shown that stress increases anxiety-like behavior and impairs memory function [7–9]. However, in these studies, exposure to the stressor was performed only during the light cycle. It is not known whether restraint during the dark cycle affects behaviors. It has been demonstrated that restraint during the light cycle induces corticosterone release and increases expression of the neuronal activity marker c-Fos in the brain, indicative of hypothalamic–pituitary–adrenal (HPA) axis activation [10–12]. Restraint stress also activates the serotonin (5-HT) system in the brain, which plays a major role in anxiety behaviors [13–15]. We recently found that dark cycle crowding, but not light cycle crowding, increases plasma corticosterone levels in adolescent mice [6]. In this study, we examined the effects of light and dark cycle restraint on anxiety behavior in adolescent mice to clarify the role of stress in brain development. Furthermore, we examined the effects of restraint on plasma corticosterone levels, c-Fos expression in the brain, brain levels of 5-HT and the metabolite 5-hydroxyindoleacetic acid (5-HIAA), and functional activation of the γ-aminobutyric acid (GABA) system.

2. Materials and methods

2.1. Animals and restraint stress

All animal studies were approved by the Animal Care and Use Committee of the Graduate School of Pharmaceutical Sciences, Osaka University. The experimental procedures involving the use of animals were conducted according to the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society. Every effort was made to minimize animal suffering and to reduce the number of animals used. Three-week-old male CD-1 mice (SHIMIZU Laboratory Supplies Co., Ltd., Kyoto, Japan) as adolescent mice were housed in wire-topped clear polycarbonate cages (28 cm × 17 cm × 12 cm) in groups of four animals under controlled environmental conditions (22 ± 1 °C; 12:12-h light/dark cycle, lights on at 08:00 h; food and water *ad libitum*) for 1 week before use in experiments. In some experiments, 9 week-old male CD-1 mice were used as adult mice. Restraint stress was applied as described previously using a ventilated 50 ml polypropylene tube [11]. For acute restraint stress, mice were individually restrained for 3 h starting from 10:00 (light cycle) or 22:00 (dark cycle) in novel cages, and then returned to the home cages. Non-stressed control mice were placed into novel cages and deprived of food and water at the same time that stressed mice underwent restraint, and then returned to the home cages. The experiments were carried out 30 min after exposure to restraint during either the light or dark cycle unless otherwise specified. For chronic restraint stress during the dark cycle, mice were individually restrained for 3 h (22:00–1:00 h) daily for 2 weeks. The mice were returned to the home cages immediately after exposure to restraint stress. The experiments were carried out 30 min or 12 h after the last restraint exposure.

2.2. Measurement of spontaneous locomotor activity

Locomotor activity was measured using a digital counter system with an infrared sensor (Supermex®, Muromachi Kikai Co., Ltd., Tokyo, Japan) [16,17]. Each mouse was placed individually in a novel clear polycarbonate cage (28 cm × 17 cm × 12 cm), and locomotor activity was recorded for 90 min. Each sensor monitors motion in multiple zones of the cage through an array of Fresnel lenses from a position up above the cage. So movement in X, Y and Z can be covered. The sensor head consists of paired infrared pyroelectric detectors which measure the radiated body heat of the animal. The sensor's output signals representing magnitude of the animal's movement are digitally converted, stored in the interface memory and transferred to a personal computer.

2.3. Contextual fear conditioning test

The contextual fear conditioning test was performed as previously reported [6,18]. Each mouse was placed in the conditioning chamber (30 cm × 30 cm × 30 cm, transparent Plexiglas enclosure with stainless-steel shock grid floor; Neuroscience, Inc., Tokyo, Japan) and allowed to acclimatize to the new environment (conditioned stimulus) for 2 min after which time animals received a 5-s footshock (0.8 mA) (unconditioned stimulus). This procedure was repeated five times at 15-s intervals. At the end of the last context-shock pairing, the mice were left in the chamber for a further 15 s, after which they were returned to the home cages. The behavior of the mice was recorded using a digital camera, and the freezing time during the acclimation and inter-trial intervals was measured by a well-trained observer blinded to the stress exposure. The percent of freezing during the 2-min acclimation period or during each 15-s inter-trial interval was calculated. Freezing behavior was defined as the complete absence of any movement except for respiration. Mice were placed individually into the same conditioning chamber 24 h after the conditioning and left there for 2 min. The total freezing time was measured, and the percent freezing over the 2-min period was calculated.

2.4. Elevated plus-maze test

The elevated plus-maze test was performed as previously reported [6,19]. The apparatus—consisting of two open arms (25 × 8 cm) and two enclosed arms (25 × 8 cm, surrounded by a 20-cm-high opaque wall)—was elevated 50 cm from the ground (BrainScience·Idea Co., Ltd., Osaka, Japan). Each mouse was placed on the central platform with its head facing an open arm, and was allowed to move freely for 5 min under dimly light conditions (15 lx). The performance of the mouse over a 5-min period was videotaped using a digital camera, and then subsequently scored by a well-trained observer blinded to the stress exposure. Arm entry was defined as all four paws entering an arm. The time spent in various sections of the maze (open arms, closed arms, central platform) and the numbers of open and closed arm entries were recorded. The following parameters were calculated: (i) ratio of time spent in the open arms (time spent in the open arms/time spent in the open and closed arms); (ii) ratio of open arm entries (open arm entries/total entries); (iii) total arm entries (entries into open and closed arms).

2.5. Pentobarbital-induced sleep

Pentobarbital-induced sleep in mice was measured as previously reported [20]. Mice were intraperitoneally injected with pentobarbital (50 mg/kg; Nacalai Tesque, Kyoto, Japan), and subsequently, sleeping time was taken as the period between the loss of the righting reflex and its return. Latency to sleep was also measured.

2.6. Corticosterone assay

Plasma corticosterone levels were measured as previously reported [6]. Immediately after restraint for a 3-h period during the dark or light cycle, each mouse was sacrificed by rapid decapitation,

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