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Research report

Social crowding in the night-time reduces an anxiety-like behavior and increases social interaction in adolescent mice

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HIGHLIGHTS

- Night-time crowding (20 mice/cage) over 2 weeks induced anxiolytic effects.
- These anxiolytic effects were not observed following daytime crowding.
- These anxiolytic effects were observed in adolescent but not adult mice.
- Acute night-time crowding increased plasma corticosterone levels.
- Night-time crowding in adolescence is beneficial to brain functions.

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ABSTRACT

Rearing in crowded conditions is a psychosocial stressor that affects biological functions. The effects of continuous crowding for many days have been studied, but those of crowding over a limited time have not. In this study, we examined the effects of night-time or daytime crowding over 2 weeks on behavior in adolescent and adult mice. Crowding (20 mice/cage) in either the night-time or daytime did not affect locomotor activity in the open field test or cognitive function in the fear conditioning test. In contrast, night-time crowding, but not daytime crowding, had an anxiolytic effect in the elevated plus-maze test and increased social interaction in adolescent mice, but not in adult mice. The first night-time, but not daytime, crowding increased plasma corticosterone levels in adolescent mice, although night-time crowding over 2 weeks did not affect the corticosterone levels. Furthermore, no significant effects of the first crowding were observed in adult mice. In a second crowding condition (six mice/small cage), the anxiolytic-like effects of night-time crowding and the change in plasma corticosterone levels were not observed, suggesting that the density of mice is not important for the behavioral consequences of crowding. Night-time crowding did not affect neurotrophic/growth factor levels and hippocampal neurogenesis in adolescent mice. These findings suggest that night-time crowding leads to anxiolytic-like behaviors in adolescent mice, and imply that night-time crowding stress in adolescence may be beneficial to brain functions.

1. Introduction

Voluntary exercise produces antidepressant- and antianxiety-like behaviors in mice [1], whereas maternal separation or social isolation during development causes increased aggressiveness, anxiety-related behaviors, and hyperlocomotion [2–5]. We have recently found that encounter stimulation induces abnormal behaviors in isolation-reared mice and the induction of some abnormal behaviors is triggered by activation of the serotoninergic system [6]. Furthermore, we found that housing condition including social isolation affects the expression of abnormal behaviors in mice lacking the pituitary adenylate cyclase-activating polypeptide [7]. These findings suggest that environmental factors play a key role in the development of brain functions [8–10]. Rearing in crowded conditions, an opposite condition form isolation rearing,

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is also considered to be an environmental stressor. Crowding stress decreases body weight gains [11–13] and increases adrenal weight and blood corticosterone levels [12]. Furthermore, crowding causes a depressive-like effect [14], anxiety-like behavior [15–17], and alters sensitivity to naloxone [18], morphine [19], and ethanol [20]. Taken together, it is likely that rearing in crowding conditions alters brain function, resulting in changes in behaviors and responses to drugs, but the mechanisms are not known.

In previous studies of crowding stress, rodents were exposed to a crowded condition continuously. The crowded condition is considered a model of psychosocial stress, but no previous study has compared the effects of night-time and daytime crowding stress on behavior. Studying the effects of crowding in specific periods of the day should allow for a better understanding of role of environmental factors in brain development. Environmental factors such as an enriched environment [21–23] and physical exercise [24–26] increases the levels of neurotrophic/growth factors such as brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), insulin-like growth factor I (IGF-1), and hippocampal neurogenesis. Furthermore, Parihar et al. [27] have recently shown that predictable, unlike unpredictable, chronic mild stress for 4 weeks decreases depressive- and anxiety-like behaviors, enhanced memory function, and increased hippocampal neurogenesis. However, it is not known whether these factors or whether neurogenesis is involved in the effects of crowding stress.

The present study compared the effects of daytime and night-time crowding over 2 weeks on locomotor activity, social interaction, anxiety-like behavior, and cognitive function in adolescent and adult mice. Furthermore, we examined the effects of night-time crowding on brain neurotrophic/growth factor levels and hippocampal neurogenesis.

2. Materials and methods

2.1. Animals, rearing conditions and experimental schedules

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- or seven-week-old male CD-1 mice (SHIMIZU Laboratory Supplies Co., Ltd., Kyoto, Japan) were commercially purchased and 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 0800 h; food and water *ad libitum*) for 1 week before use in experiments.

For experiments using adolescent mice, 4-week-old mice were divided into three different crowding groups and standard housing group (Fig. 1). The mice in the crowding group were housed in groups of 20 mice/cage during either the daytime (8:00–20:00 h) or night-time (20:00–8:00 h) in wire-topped opaque polypropylene cages (28 cm × 17 cm × 12 cm) or in groups of six mice/cage during the night-time (20:00–8:00 h) in wire-topped small opaque polypropylene cages (17 cm × 10 cm × 10 cm) over 2 weeks before the initiation of behavioral assessments, whereas the control group continued to be housed under standard housing conditions (four mice/cage measuring 28 cm × 17 cm × 12 cm) but the mice were housed with different cage mates during either the daytime (8:00–20:00 h) or night-time (20:00–8:00 h). For experiments using adult mice, 8-week-old mice were housed either in groups of four mice/cage (standard housing group) in wire-topped clear polycarbonate cages (28 cm × 17 cm × 12 cm) or 20 mice/cage (crowded housing, 285.6 cm^3/mice) in same-sized wire-topped opaque polypropylene cages in either the daytime (8:00–20:00 h) or night-time (20:00–8:00 h) over 2 weeks before the initiation of the behavioral assessments. The mice were returned to the home cages immediately after exposure to the crowding or control condition during either the daytime or night-time. In the home cage, the mice were exposed to the same mates. These rearing conditions continued until the end of behavioral assessments.

Body weight of mice was measured three hours after exposure to the crowding or control condition during either the daytime or night-time. Body weight was recorded before and at 3, 7, 10, and 14 days after the initiation of crowding. For the behavioral analyses, animals were divided randomly to two experimental groups. Mice in group 1 were subjected to the locomotor activity test (day 1) and the elevated plus-maze test (day 3) with a 1-day interval between each test. Mice in group 2 were subjected to the social interaction test (day 1) and the contextual fear conditioning test (day 3 and 4) with a 1-day interval between each test. All behavioral tests were performed at 13:00–19:00; that is, the tests started at five hours after exposure to night-time crowding or at 17 h after exposure to daytime crowding. The housing condition continued except of the time of the behavioral tests. The contextual fear conditioning test was performed without exposure to the crowding condition after the housing. None of the mice was subjected to the same behavioral test twice, so behavior at 6 and 10 weeks of age was evaluated using different animals. For corticosterone assay, measurement of neurotrophic/growth factors and immunohistochemistry, different mice from those used in behavioral studies was used in each assay. The animal numbers in this study are shown in Table 1.

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) [6,28]. Each mouse was placed individually in a novel clear polycarbonate cage (28 cm × 17 cm × 12 cm), and then locomotor activity was recorded for 90 min.

2.3. Elevated plus-maze test

The elevated plus-maze test was carried out according to a method previously reported [29,30]. 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 dim light conditions (20 lx). The performance of the mouse for 5 min was videotaped using a digital camera, and then subsequently scored by a well-trained observer blinded to the rearing condition. Arm entry was defined as all four paws into an arm. The time spent in various sections of the maze (open and closed arms, central platform) and the number 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 to open and closed arms).

2.4. Reciprocal social interaction test

The subject mouse and a novel male CD-1 mouse from a different litter at similar age that housed under standard housing condition (four mice/cage) were introduced simultaneously into a novel clear polycarbonate cage (28 cm × 17 cm × 12 cm). Each pair was socially naïve to each other before the test session. The interaction between