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Age dependent effects of space limitation and social tension on open-field behavior in male rats

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Abstract

Cage stocking density can be manipulated by changing in cage size and group size in the cage. The effects of these two factors were investigated in three developmental stages in male rats: juvenile, post-pubertal, and adult. An open-field test was conducted to measure exploratory behavior which was influenced by levels of activity, anxiety, and exploration. When the cage size was decreased, juvenile rats displayed decreased locomotion and lower propensity for exploration, whereas such changes were not evident in post-pubertal rats. When the group size was increased, adult rats exhibited higher locomotion. However, these factors did not affect risk assessment behavior of rats in these developmental stages. Thus, it appears that the effect of stocking density differs depending on the developmental stage of the animal: Juvenile rats increased anxiety following limiting space, whereas adult rats increased activity following increase in social tension.

Keywords: Stocking density; Crowding; Social development; Exploration; Locomotion; Open-field; Rats

1. Introduction

Cage stocking density is an important factor for experiments using animals, which relates with crowding effect that is considered to be a model of chronic social stress [1–3]. In experimental paradigms, stocking density can be manipulated by changing the floor area per animal or the number of animals per cage [4,5]. Animals, including mice [6,7], domestic hens [8], pigs [9], domesticated cats [10], rhesus monkeys [11], and primates [12], have been reported to demonstrate a variety of behavioral and physiological changes depending on their stocking density.

In the studies by Calhoun [13,14], rats reared in higher stocking density showed a variety of socio-pathological states (behavioral sink) including abnormalities of social and sexual behaviors, and decreased fertility rates. In early studies, rearing in higher stocking density had been assumed to disturb the appropriate establishment of social relationships by excessive physical contacts with conspecifics [15,16]. Male rats have shown increased aggression as a function of increased stocking density [15,17,18], although some studies have reported no effect of stocking density on aggression [19,20] or even a reverse effect [21]. Similarly, the findings of previous studies investigating the effect of stocking density on exploratory behavior of animals in novel environments such as an open-field have remained equivocal. For example, one study reported that animals showed increased activity with increasing stocking density [22], whereas other studies reported no behavioral differences dependent on stocking density [23], or even reported a reverse effect [24,25]. These discrepancies may be attributable to not distinguishing between cage size which associates with limiting space and group size which relates with social tension in the experimental design.

Taking these two factors into consideration, Van Loo et al. [5] found that level of aggression was influenced substantially by group size, and suggested that the increased

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frequency of daily territorial confrontations is the underlying factor contributing to the behavioral changes associated with increasing stocking density [6]. When adult male rats have been exposed to repeated confrontations with novel males in their home cages, all animals display increased aggression and activity [6,26]. In contrast, adult female rats, which do not show male-like territorial defenses toward intruders [27], do not exhibit behavioral changes depending on stocking density in the cage [3,28]. In long-term observation of a situation with higher stocking density, Henry and Stephens [29] found that all rats showed enhanced sympatho-adrenal responses, such as a chronic increase in blood pressure accompanied with increased aggression and activity. These physiological and behavioral changes are similar to those found in subdominant males [30 31], suggesting that animals reared in higher stocking density are exposed to high social tension similar to those experienced by subdominant animals.

Male rats display adult-like social behaviors that are associated with the establishment of a dominance territory after the post-pubertal stage [32–35], and the peak in agonistic interactions with male conspecifics occurs after the age of 100 days [36,37]. In contrast, rats in the juvenile stage show frequent play behavior, but do not exhibit a social dominance hierarchy or territory [36,38,39]. In a previous study [39], adult male rats displayed differences in exploratory behavior that were associated with the establishment of an adult dominance hierarchy [see also Ref. 40]. As such, if the emergence of a dominance territoriality is a determinant of the effects of stocking density [5,41], adult animals should show differences in exploratory behavior substantially with increasing group size.

The present study investigated whether the effects of cage size and group size on open-field behavior in male rats during three developmental stages (juvenile, post-puberty, and adults) are due to excessive physical contacts or to the emergence of a dominance territoriality. The present experiment was conducted using a three-way factorial design, with the between subject factors of Age (40, 65, or 130 days) which reflects the developmental stages of juvenile non-territoriality, post-pubertal or adult territoriality, Cage Size (large or small) which reflects the factor of limiting space, and Group Size (two or four rats per cage) which reflects the factor of social tension.

The open-field behavior of animals is known to be influenced by several factors, such as general activity, anxiety and exploration [cf., [42–44]]. Moreover, exploratory behavior of rats can be divided into two types: an active pattern in which rats actively approach a novel stimulus, and a passive pattern (risk assessment behavior) in which rats search potential threats in a novel environment with less ambulation [30,39,40]. The current study employed an open-field apparatus that was divided by an inner wall into a brightly lit center square and a dimly lit peripheral alley. In this paradigm, activity is reflected in the amount of ambulation in the peripheral area, while exploration and anxiety are reflected in the approach behaviors to the phobic center area. The active pattern of exploratory behavior, which was primarily reflected by anxietic property of animals, was assessed as the amount of time spent in the center area, and the passive pattern of exploratory behavior, which was reflected by level of exploration, and was measured as the number of stretch attend postures for the center area [39,40].

2. Materials and methods

2.1. Subjects and rearing conditions

Ninety-six male Wistar rats, bred and reared in the colony room of the Department of Psychology at Nagoya University, were used as subjects. After weaning at the age of 22–23 days, which was determined based on the growth of mean body weights for each litter, each animal was reared with other male littermates in groups of two to four in a cage measuring 36×30×18 cm (l×w×h). At the age of 26 days, the animals were randomly distributed into one of twenty groups (N=8 for each group). The sizes of the opaque plastic cages used in the present experiment were as follows: Small Cage I, 24×17×12 cm (l×w×h), Small Cage II, 31×21×14 cm (l×w×h), Small Cage III, 26×30×18 cm (l×w×h), Large Cage I, 36×30×18 cm (l×w×h), Large Cage II, 45×36×18 cm (l×w×h), and Large cage III, 60×36×18 cm (l×w×h). The tops of these cages were covered with lids of stainless steel wire grid. The floors of the cages were covered with sawdust that was changed every week.

All the animals to be tested at the age of 40 days were divided into groups at the age of 26 days, with rats in Groups S2 and S4 reared in Small Cage I, and those in Groups L2 and L4 reared in Large Cage I. All the subjects to be tested at the age of 65 days were reared in pairs in Large Cage I until the age of 45 days, and then were reared in Large Cage II. At the age of 51 days, they were divided into the following four experimental groups: Groups S2 and S4 in Small Cage II and Groups L2 and L4 in Large Cage II. All the rats to be tested at the age of 130 days were reared in pairs in Large Cage I until the age of 45 days, and then reared in Large Cage II until the age of 90 days. Thereafter, the rearing cages were changed to Large Cage III. At the age of 116 days, they were divided into the following four groups: Groups S2 and S4 in Small Cage III and Groups L2 and L4 in Large Cage III. Each animal in Groups S2 and L2 was raised in pairs with a male littermate, and those in Groups S4 and L4 were raised in a group of four male littermates. These cage sizes were chosen based on the ratio between the mean body lengths of the subjects (length×width) and the area of floor space per animal in the cages for each age of the animals to maintain a similar ratio of body size to floor space based on the age/size of the rats. The diagram of these housing procedures is presented on Fig. 1.