Effects of the housing system and environmental enrichment on social dominance in laboratory male rats

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Abstract

In laboratory rats, dominance manifests as agonistic behaviour that damages social bonds between individuals. In this study, the effect of the housing system and environmental enrichment on the social dominance in male Wistar rats was assessed in the social dominance tube test. Rats were housed in different housing systems (individual vs. social housing, with or without enrichment) from weaning and tested at the age of 7 weeks. In each test, two rats from different housing systems were released into opposite ends of a narrow tube and the rat that forced its opponent out of the tube was declared the winner (the more dominant animal). In this way, all possible combinations of housing systems were tested and number of wins were recorded and percentage of the total number of matches was calculated. The results show that environmental enrichment suppresses ($P < 0.001$) dominant behaviour in individually housed rats while no such effect was seen in socially housed male rats ($P = 0.532$). However, social housing combined with enrichment was more effective in reduction of dominant behaviour compared to only providing enrichment for individually housed rats. Reduction of variability in the manifestations of dominant behaviour is important in animals used for experimental purposes from the perspective of greater homogeneity of animals, which ensures obtaining valid research results and at the same time better living conditions for laboratory animals.

Group housing, isolation, enriched cage, rodent, behaviour, tube test

Social housing provides animal welfare benefits for social animals (Malá et al. 2023). However, stable hierarchy is essential in minimizing unnecessary fights among group members (Fan et al. 2019; Popelková et al. 2022). Social or dominance hierarchies are observed in many animal societies where group members maintain relatively dominant or subordinate statuses (Fan et al. 2019). Dominance is of a great importance from the point of view of the social structure. It controls not only the course of social interaction, but also has an impact, for example, on where and when an individual will move or rest, or on its reproductive success. In rats, dominant behaviour occurs mainly in males. Males usually compete for a higher rank and its achievement significantly affects the quality of their life (Fernald 2014). In females, dominance is also manifested, but under different sex-specific stimuli and endocrine conditions (DeBold and Miczek 1984). Animals kept in laboratory facilities may be subjected to agonistic outbursts of animals they are housed with. According to Gardner (2001), social defeat leads to subordination: the dominant animal takes priority in gaining access to valued resources (Berdoy et al. 1995). Social dominance also affects exploratory behaviour as dominant and subordinate animals differ in patterns of exploratory behaviour (Arakawa 2005). In the case of social housing of animals, a form of certain order of dominant behaviour results from the interactions

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between animals. According to Scott (1966), however, rats and mice share an inability to establish a complex dominance hierarchy in which fighting is reduced to threat and avoidance. Nevertheless, Nádeníček et al. (2022) have documented that social housing reduces anxiety and depressive-like behaviours in juvenile rats.

In laboratory rats kept for experimental purposes, dominance manifests as agonistic behaviour that damages social bonds between individuals kept in the same cage (Hurst et al. 1999; Van Loo et al. 2002), which in turn leads to physical impairment and associated social stress (Hurst et al. 1996). Agonistic interactions in rodents can be reduced by environmental enrichment (Armstrong et al. 1998; Kaliste et al. 2006). The impact of environmental enrichment on the reduction of agonistic behaviour has also been documented in other animal species, such as pigs (O'Connel and Beattie 1999), poultry (Gvaryahu et al. 1994) or captive primates (Honess and Marin 2006). In contrast, some studies have suggested that enrichment may lead to increased aggressive behaviour in male mice, although the effect of the mouse strain and the housing system must be also considered (Marashi et al. 2004).

Therefore, the aim of this study was to determine the effect of the housing system and environmental enrichment on the social dominance in laboratory male rats.

Materials and Methods

Animals and their housing

The objects in this study were male Wistar rats (*Rattus norvegicus*). All experimental animals were kept with their mothers since birth until weaning in the same room under the same conditions (the difference in the time of birth for individual litters was 1 to 2 days). The young were weaned at the age of 21–23 days and their sex was determined. A total of 48 males were randomly selected and housed in a room accredited for housing laboratory animals under standardized conditions: 12/12 h light cycle (light/dark), 21.0 ± 1.2 °C temperature, 78–87% relative humidity. They were provided with standard pellet feed (Altromin Spezialfutter GmbH & Co., Lage, Germany) and water *ad libitum*. For the purposes of the study, the rats were randomly divided into four housing systems with 12 individuals each: 1) individual housing without environmental enrichment, 2) individual housing with environmental enrichment, 3) social housing without environmental enrichment and 4) social housing with environmental enrichment. Individually housed rats were kept in standard 40 × 26 × 20 cm (l × w × h) cages. Socially housed rats were housed in groups of six individuals per cage with the dimensions of 120 × 80 × 80 cm (l × w × h). For the groups of both individually and socially housed rats with environmental enrichment, enrichment was provided and changed or rearranged at regular intervals according to the protocol described by Arai et al. (2009). As enrichment, the rats were presented with orally modifiable material in the form of wooden as well as plastic objects, swings, climbing frames, underground and elevated shelters. Furthermore, various types of wood-based bedding were used. Enrichment items were of different shapes and colours to stimulate active exploration (Li et al. 2013).

Social dominance tube test

The social dominance tube test is used to assess social dominance in rats. In the test, the outcome of a forced encounter between two unfamiliar animals is evaluated (Spencer et al. 2005; Garfield et al. 2011; Wang et al. 2011). In this study, the social dominance tube test was used in male rats to determine whether the housing system and environmental enrichment of their environment suppresses dominant behaviour. The test was performed at the age of seven weeks. A transparent tube with a total length of 1.5 m and a diameter of 6 cm was used. The width of the tube guaranteed that only one individual could pass through and could not change direction. The test was preceded by an acclimatization process to ensure easy passage through the tube by the rats. Two days before the test, each rat was individually placed with its head inside the tube twice, after crossing to the other side of the tube, the animal was returned back to its home cage. During the test, two rats from different housing systems were simultaneously released into opposite ends of the tube and their interactions were observed for 2 min. The observer recorded which rat was pushed out, i.e. the test ended once one of the rats had all four paws outside the tube. The rat that forced its opponent out of the tube within 2 min was considered the winner. Each pair of rats was tested twice and their starting positions were switched. All possible combinations of housing systems were tested in this way. In cases when no rat moved forward within 2 min, the test ended in a draw. The number of wins was reported as percentage of the total number of matches between the rats from the two housing systems tested (n = 48). After each test, the tube was cleaned with a 70% ethanol solution.

Statistical analysis

The percentage of forwards was calculated from the number of wins obtained by a rat from a certain housing system over a rat from another housing system in the social dominance tube test. Data were statistically analysed

using Unistat v. 6.5 (Unistat Ltd., London, UK) and the frequencies were compared applying the Chi-square test with Yates correction to evaluate statistical significance within a 2 × 2 contingency table procedure. A P value ≤ 0.05 was considered significant.

Results

A significant effect of environmental enrichment on social interactions was seen in individually housed male rats. Individually housed rats with access to enrichment showed a highly significant ($P < 0.001$) reduction in the percentage of forwards compared to rats that were housed individually without enrichment (Fig. 1). In socially housed rats, no effect of environmental enrichment was found on the percentage of forwards in the social dominance tube test.

No difference ($P = 0.522$) was found between non-enriched individual and social housing of rats in the percentage of forwards in the social dominance tube test. In contrast, when enrichment was provided, socially housed rats showed a highly significant ($P = 0.008$) reduction in the percentage of forwards compared to individually housed rats (Fig. 2).

The results of the social dominance tube tests cross-comparing rats in different housing systems with or without enrichment are shown in Fig. 3. Individually housed rats without access to enrichment showed significantly higher ($P = 0.014$) percentage of forwards compared to socially housed rats with enrichment. No difference ($P = 0.307$) was found between individually housed rats with access to enrichment and socially housed rats without enrichment in the percentage of forwards in the social dominance tube test.

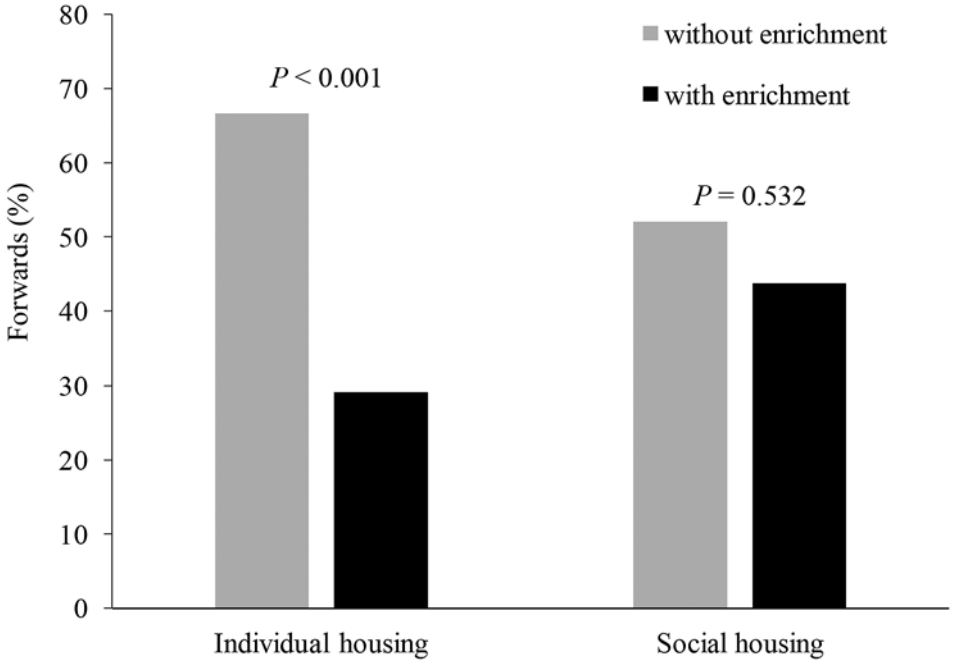

Fig. 1. Effects of environmental enrichment in male rats housed individually or socially as shown in the social dominance tube test.

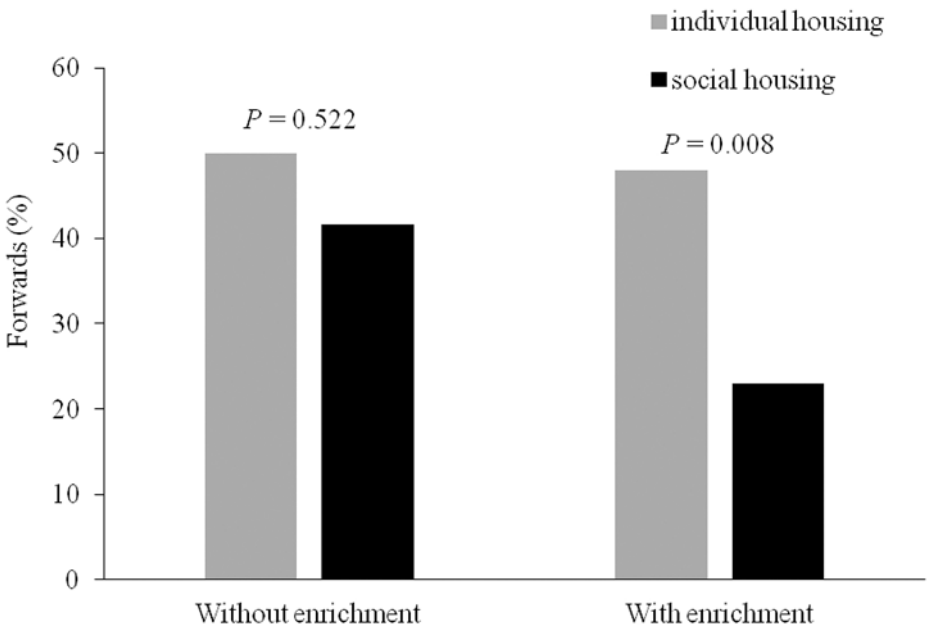

Fig. 2. Effects of housing (individual vs. social) in male rats housed with or without enrichment as shown in the social dominance tube test

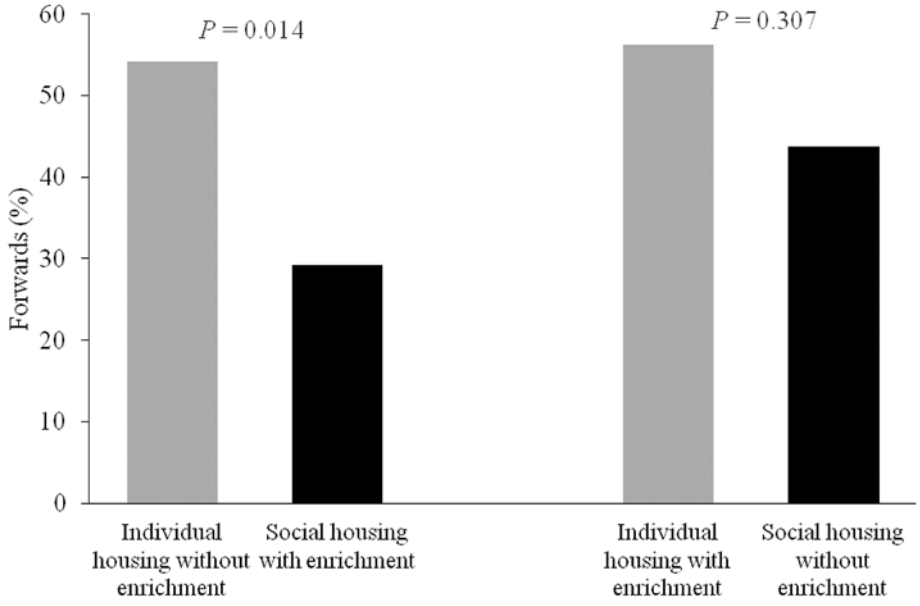

Fig. 3. Effects of housing (individual vs. social) and environmental enrichment in male rats as shown in the social dominance tube test

Discussion

Social hierarchy determines the social position of animals living in groups, affecting their physical and emotional health. An animal that gains a high social rank in a group is able to maximize control over access to resources such as food or water (Korzan et al. 2006). High hierarchical status generally translates into better physical condition and higher survival rates, while low social status is usually associated with increased levels of stress, both in animals and humans (Blanchard et al. 1995; Sherman et al. 2012). However, in animals kept in captivity, group composition and changes in it result from the keeper's decisions, and stable social hierarchy often cannot be established. The tendency towards dominant behaviour is undesirable and may become a welfare issue. This study aimed to determine the factors affecting the social dominance in laboratory male rats, namely the housing system and environmental enrichment. To assess dominant behaviour, the tube test was used. It is used in preclinical studies in rodent models for a better understanding of the effects of an individual's social status on other behaviours and physiological processes (Fulenwider et al. 2021). Our results show that the housing system affected the manifestation of dominant behaviour against an unknown individual. In individually housed rats, the provision of enrichment led to a suppression of dominant behaviour. Rats reared in enriched cages after weaning were more frequently (67%) forced out of the tube by the rats reared in isolation without enrichment than the other way round. Providing shelter, bedding, and other enrichment items allows animals to escape danger or hide from other aggressive animals, whereas animals kept in isolation in a bare environment have no such options, which may result in frustration (Hurst et al. 1999; Sørensen et al. 2004; Brandão and Mayer 2011) and a more pronounced reaction when encountering an unknown individual. In contrast, experience with enrichment items could have caused greater willingness to leave the tube and look for a safe place elsewhere instead of continuing in agonistic interaction. In addition, provision of enrichment items in the form of orally modifiable objects (e.g. wooden blocks or wood wool) enables species-specific behaviours such as gnawing and may reduce the motivation for agonistic interactions resulting from unsatisfied needs (Orok-Edem and Key 1994; Bardi et al. 2016).

Suppressed dominant behaviour was also observed in rats housed in a group in the enriched cage compared to rats housed individually in enriched cages, and in rats housed socially with enrichment compared to rats housed in isolation without enrichment. The results suggest that the combination of social housing with the provision of environmental enrichment is the most effective in reducing dominant behaviour. Group housing can also be considered as environmental (social) enrichment (Stewart 2017). However, in our study, social housing in cages without other enrichment did not result in suppressed dominant behaviour compared to rats housed in isolation. Even when rats were housed individually in enriched cages, there was no difference in their dominant behaviour compared to rats housed socially without enrichment. On the other hand, the combination of social housing and environmental enrichment clearly leads to a decrease in social dominance, as shown when comparing rats housed socially in enriched cages with rats housed in isolation in enriched cages in the social dominance tube test. The provision of enrichment in socially housed rats was not significant in terms of its impact on social dominance in our study, no difference was found in the tube test. In contrast, Abou-Ismail (2011) reported lower levels of both successful aggressive and successful defensive bouts in rats in the enriched cages compared to rats in the standard cages. The cage modification regimen implemented in their experiment differed as well as the number of rats in the group and methods of testing of agonistic behaviour and dominance. In our study, the provision of enrichment led to a reduction in dominant behaviour only in individually housed rats. We assume that in socially housed animals certain interactions take place between individuals, whether

in the form of play, jumping or grooming, which are frequent activities of young rats, when a hierarchy and a dominant position are formed in the group (Hurst et al. 1996; Berdoy and Drickamer 2007; Wang 2014). Although some studies report that placing enrichment items in the cage can destabilize social hierarchy by being perceived as a resource to be defended (Howerton et al. 2008; Shemesh et al. 2013), no difference was found in the dominant behaviour of socially housed rats depending on the provision or non-provision of enrichment in our study. Similarly, Abou-Ismail (2011) did not find any negative changes in agonistic behaviour of rats kept in groups in enriched cages. Considering physiological, psychological, developmental and therapeutic advantages that housing in enriched conditions may provide, the authors recommended enhancing the complexity of cages of laboratory rats as an improvement of welfare.

In conclusion, the housing system and environmental enrichment affect the social behaviour of laboratory rats, namely agonistic interactions and dominance. In terms of reducing dominant behaviour, group housing with access to environmental enrichment appears to be particularly effective compared to individual housing. The reduction of variability in the manifestations of dominant behaviour is important in animals used for experimental purposes from the perspective of greater homogeneity of animals, which ensures obtaining valid research results and at the same time, better living conditions of laboratory animals.

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