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The effects of cage enrichment on agonistic behaviour and dominance in male laboratory rats (*Rattus norvegicus*)

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ABSTRACT

This experiment was carried out to investigate the effects of enriching laboratory cages on agonistic interaction and dominance of rats. In a series of three replicates, 48 rats were housed in groups of four in either 'standard' or 'enriched' cages for 6 weeks. Successful aggressive and defensive behaviour that ended up in a clear winner and loser were sampled in the first hour of the dark phase of the light/dark cycle every other week. Rats in the 'complex' cages showed lower levels of both successful aggressive and successful defensive bouts compared to rats in the 'standard' cages. Enriching cages of laboratory rat did not change the social order of the animals in the cage. Thus, enhancing the complexity of cages of laboratory rats by the particular cage modification regimen implemented in this experiment could be considered enrichment and could therefore result in an improvement of welfare in these animals.

1. Introduction

Experiments on laboratory rodents have demonstrated wide effects of environmental modifications, including physiological (Belz et al., 2003; Chamove, 1989; Roy et al., 2001), psychological (Chamove, 1989; Patterson-Kane et al., 1999), developmental (Davenport et al., 1976) and therapeutic effects (Hockly et al., 2002; Passineau et al., 2001).

In addition to the physiological, psychological, developmental and therapeutic advantages that housing in enriched conditions may provide, research has elucidated behavioural benefits from being housed in enriched environments (Armstrong et al., 1998; Chamove, 1989; Orok-Edem and Key, 1994; Van Loo et al., 2002). An important behaviour in group-housed laboratory animals is agonistic behaviour. Damaging social behaviour between conspecifics, such as excessive agonistic behaviour, is a common problem related to housing male laboratory rodents in captivity (e.g. Hurst et al., 1999; Van Loo et al., 2002), leading to physical damage and associated social stress and poor welfare (e.g. Hurst et al., 1996, 1999).

It is interesting to note that there is a conflict between the results of experiments regarding the effects of environmental enrichment on agonistic interaction in laboratory rodents. Some experiments reported that the addition of environmental enrichment can, sometimes, reduce excessive aggression between rodents kept under standard unenriched housing conditions (Chamove, 1989; Armstrong et al., 1998; Orok-Edem and Key, 1994; Van Loo et al., 2002; Kaliste et al., 2006). Similar results of reduced aggressive encounters between members of group-housed animals in enriched housing conditions have been reported in other animals; pigs (O'Connell and Beattie, 1999), laying hens (Gvaryahu et al., 1994) and captive primates (Kitchen and Martin, 1996). Others have reported no differences in levels of agonistic interaction between animals housed in standard versus enriched laboratory cages (Marashi et al., 2004) due mainly to the use of low animal number per cage and the kinship of animals.

However, in contrast to these findings, there are data that also reported an increase in agonistic behaviours between rodents housed in groups in 'enriched' housing conditions (Haemisch and Gartner, 1997; Haemisch et al., 1994; McGregor and Ayling, 1999; Nevison et al., 1999; Van Loo et al., 2002; Kaliste et al., 2006). This increase in the agonistic interaction between animals housed in enriched cages has been reported in laboratory mice particularly and is either due to the encouragement of territorial behaviours (Haemisch and Gartner, 1997; Haemisch et al., 1994), rigidity and lack of manipulability of the objects that decreases the ability of the animal to control its environment (e.g. Van Loo et al., 2002) or due to the exposure of vulnerable body parts such as tails to biting (Nevison et al., 1999; Van Loo et al., 2002). The increase in the level of agonistic interaction might counteract the general goal of enrichment to improve animal welfare since high levels of aggression may cause physical and/or psychical injury.

It is therefore also interesting to emphasize that the effects of different environmental modification regimens depend mainly on the species and strains of the animals experiencing it (Chapillon et al., 1999; Nevison et al., 1999; Kaliste et al., 2006). Scott (1966) reported that agonistic interaction in rats and mice are

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different and that both species are alike in their inability to form complex dominance hierarchies in which fighting is reduced to threat and avoidance. Van de Weerd et al. (1994) reported that environmental "enrichment" may actually increase the level of anxiety for some strains of laboratory mice. Similarly, Nicol et al. (2008) showed that changing enrichments regularly can adversely affect some strains but not others.

To the authors' knowledge, a vast majority of the experimental work carried out on the effects of environmental modification on the welfare of laboratory rodents was done using experimental designs that supplied the laboratory cages with a single enrichment item such as gnawing materials (Orok-Edem and Key, 1994), shelters (Townsend, 1997) nesting materials (Haemisch and Gartner, 1997; Haemisch et al., 1994; Van Loo et al., 2002) or foraging substrates (Johnson et al., 2004). There is a preliminary evidence from research that increasing the degree of complexity of laboratory environment (extent of enrichment) may increase the effects of enrichment and therefore improve the welfare of animals experiencing it (Marashi et al., 2004). It is therefore not known how increasing the complexity of laboratory cages, by providing multiple physical structures, can affect some key behavioural patterns indicative of welfare in laboratory rodents such as inter-male aggression.

Another major factor that determines how a particular cage modification regimen produces a change in behaviour of the animals experiencing it is whether the regimen relies on supplying items that remain unchanged throughout the study (reintroduced to the cages uncleaned after cage cleaning every week) or items that are replaced regularly during the study (replaced with exactly the same new and clean items after cage cleaning every week). There is an evidence that with some territorial species of laboratory rodents such as mice, the use of nesting material as enrichment could improve the welfare of mice (Van Loo et al., 2004a) providing that they are transferred during cage cleaning (Van Loo et al., 2004b).

When social animals are housed together, a form of dominance order emerges as a result of the interaction between them. That social interaction between animals discharges a dominant and subordinate animal(s). It has been shown that the dominant animal is the one that has the priority in gaining access to the valued resources or who supplants its opponent and remove it away when they meet (e.g. Berdoy et al., 1995; Hurst et al., 1996).

Despite that clear way of assigning dominance within group-housed animals, other methods have also been used in experimental work. One extensively used method is the outcome of the agonistic interaction between two animals. Takahashi (1986) described dominant rat as the one shows more offensive (aggressive) behaviours such as on- top, lateral display and biting, while the rat shows few or no offensive behaviours as a subordinate. It was also defined that a dominant rat in a colony is the male that never loses whilst the subordinate is the rat that last shows defensive behaviour at the end of social confrontation (Fokkema et al., 1995).

In dyadic interactions between rats the difference in the number of aggressive acts initiated and received has been used to classify rats into different social status (Popova and Naumenko, 1972; Militzer, 1982; Hurst et al., 1996). The same method was used, with more or less modification, to determine the dominant and subordinate animal within a colony of animals of other species such as mice (Poole and Morgan, 1976; Van Loo et al., 2000), hamsters (Huhman et al., 1990) and pigs (McGlone, 1993; Tuchscherer et al., 1998).

Despite the fact that a very large number of studies has been carried out to investigate the effects of environmental enrichment in laboratory rodents, nearly none of these research has considered whether environmental enrichment changes dominance order of animals within the cage or not. Changing the dominance order of a stable group of rats has been shown to be stressful (e.g. Burman et al., 2008) and social disorganization produced by forced contact (grouping) between unrelated individuals has been shown to induce agonistic interaction and serious fighting in laboratory rodents (Scott, 1966).

This experiment was therefore carried out to investigate the effects of a particular cage modification regimen (recruiting laboratory cages with multiple items that incorporated both renewal and cleaning every week and re-introduction uncleaned to the cage every week) on agonistic interaction of laboratory rats. Another aim of the experiment was to investigate whether increasing the complexity of cages of laboratory rats using multiple items affects the form of the dominance order within the cage. A further aim of the experiment was to study the form of dominance order in stable groups of newly weaned male laboratory rats.

2. Materials and methods

2.1. General animal housing and husbandry

Outbred newly weaned male Wistar rats (Hannover strain) purchased from Harlan Link Ltd. (Bicester, UK) were used in this study. Upon arrival to the laboratory the animals were housed in large metal cages (70 cm length ×52 cm width ×36 cm height) for 5 days allowing them to adapt to laboratory conditions. These cages were supplied with sawdust (LIGNOCEL, RS, grade 1–2) as a bedding material. All cages were kept in the same room to avoid exposing the rats to environments with potentials to differ and hence confounding room with treatment.

A pellet food (Eurodent diet 22%, 5LF5, PMI Nutrition International LLC, Brentwood, MO) and tap water were provided ad libitum and were refreshed daily. The rats were 6 weeks of age, on arrival, and weighed 45–60 g. They were maintained under an artificial 12:12 h light:dark cycle, with white light on between 1200 and 2400 and a continuous dim red lighting (two 60 W bulbs) to facilitate dark phase observation, at a temperature (20 ± 2 °C) and humidity (46% relative humidity).

Once a week, all rats were removed from their cages and rehoused in clean cages with new bedding material. The fur of each rat was marked with hair dye (Clairol Nice n'easy Natural Black) in one of four different patterns on the day of arrival to allow individual identification. These marks were refreshed after 3 weeks, allowing sufficient time after dyeing before behavioural observation to reduce any possible effects on behaviour of the dyeing process (e.g. Hurst et al., 1999). Tails were also marked, in one of eight distinguishable manners, with a permanent marker pen to provide an additional means of identification. Tail marks were renewed every week.

2.2. Housing systems

Three separate identical experiments with a duration of 5 weeks were carried out. In each experiment 16 rats were randomly allocated into groups of four and housed in polypropylene cages (48.5 cm length ×33 cm width ×21 cm height). Two groups were housed in cages with only sawdust bedding and two groups were housed in cages supplied with various enrichment objects.

1. "Standard": polypropylene cages without any additional cages structures.
2. "Complex": standard cages that were supplied with a number of additional cage structures such as gnawing objects (aspen wood blocks, wood balls and nylabones), shelter (rodent retreat), devices for climbing (ladders and ropes) and other