

EFFECTS OF CAGE ENRICHMENT ON AGONISTIC BEHAVIOUR AND DOMINANCE IN MALE LABORATORY RATS (RATTUS NORVEGICUS)

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SUMMARY

This experiment was carried out to study the effects of cages enrichment on agonistic interaction and dominance of newly weaned male laboratory rats using multiple items in the cage. In a series of three replicates, 72 rats were housed in groups of four in either 'standard' or 'enriched' cages for six 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 in the first week and every other week thereafter. Results revealed that rats in the 'enriched' cages showed lower

levels of both successful aggressive and successful defensive bouts compared to rats in the 'standard' cages. Results importantly demonstrated that enriching cages of laboratory rat does not change the social order of the animals experiencing it and that the order is almost linear. Thus, enriching conventional laboratory cages can ameliorate behavioural deficits stimulated by prolonged housing in standard cages such as frustrated social strategies and excessive agonistic behaviour and might therefore improve the welfare of laboratory rats.

Key words: Laboratory Rats, Enrichment, Agonistic interaction, Dominance score.

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).

Research has also 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).

There is a conflict between the results of experiments investigating the effects of environmental enrichment on agonistic interaction of laboratory rodents. Some experiments reported that the addition of environmental enrichment can, sometimes, reduce excessive aggression between rodents kept under standard

unenriched housing conditions (Armstrong et al., 1998; Chamove, 1989; Orok-Edem and Key, 1994; Van Loo et al., 2002). However, in contrast to these findings, there are data that also reported an increase in agonistic behaviour between animals (particularly mice) housed in groups in "enriched" housing conditions (Haemisch and Gartner, 1997; Haemisch et al., 1994; McGregor and Ayling, 1999; Nevison et al., 1999), either due to the encouragement of territorial behaviour or due to exposure of vulnerable body parts such as tails to biting wounds.

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) behaviour such as on-top, lateral display and biting, while the rat shows few or no offensive behaviour 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).

Despite the fact that a very large number of research has been carried out to investigate the effect 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).

This experiment was therefore carried out to investigate the effects of cage enrichment on agonistic interaction of rats using multiple items in the cage. Another aim of the experiment was to investigate whether enriching cages of laboratory rats affects the form of the dominance order within the cage.

MATERIALS AND METHODS

1. Animals and housing:

This experiment was carried out in the experimental animal unit, Department of Clinical Veterinary Sciences, University of

Bristol, UK, using three batches of rats. Within each batch the two experimental treatments used were replicated twice. Each batch comprised twenty-four outbred newly weaned male Wistar rats.

The rats were six weeks of age on arrival, and were randomly allocated, in groups of 6, in four large metal cages with sawdust as a bedding material till the start of the experiment. A pellet food and tap water were provided ad-libitum. Rats were maintained under an artificial 12:12 h light:dark cycle, with white light on between 1200 hr and 2400 hr and a continuous dim red lighting (two 60 watt bulbs) to facilitate dark phase observation, at a temperature of 20±2 °C.

Cages were checked daily and were cleaned out on a weekly basis. The fur of each rat was marked with hair dye in one of four different patterns on the day of arrival. These marks were refreshed after three weeks, allowing sufficient time after dyeing before behavioural observation to reduce any possible effects of the dyeing process on behaviour (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 (Burman et al., 2008). Tail marks were renewed every week.

2. Experimental design:

Rats were allocated to one of two housing systems for six consecutive weeks:

1- "Standard": polypropylene cages (48.5 cm length × 33 cm width × 21 cm height) without any additional cages structures.

2- "Enriched": standard cages that were supplied with a number of additional cage structures such as gnawing objects (aspen wood blocks, wood ball and nylabones), shelter (rodent retreat), devices for climbing (ladder and ropes) and other objects to stimulate general activity and gnawing such as crawl balls and wood balls. The description of these additional cage structures and the

frequency of their provision is presented in Table 1. In addition to the cage enrichment, foraging opportunities were also increased. This was done by mixing four intact food pellets, and four pellets that had been broken into numerous small pieces with the bedding material after the cages were cleaned every week. Foraging devices have been shown to be an effective way of enriching laboratory rat cages (e.g. Johnson et al., 2004).

Table 1: Description of the additional structures used to enrich the cages.

Structure	Number supplied	Provision and renewal	Description
Shredded paper	Handful	Throughout the experiment, renewed every week.	
Nestlets	4	Throughout the experiment, renewed every week.	5 cm × 5 cm sterilized cotton fibre pads.
Aspen wood block	1	Throughout the experiment, renewed every week.	34 mm × 70 mm long aspen wood block.
Rodent retreat (shelter)	1	Throughout the experiment, reintroduced uncleaned to the cage.	20.5 cm L × 15.7 cm W × 11.5 cm H Guinea pig huts, red-tinted.
Percher	1	Throughout the experiment, reintroduced uncleaned to the cage.	Pure cotton rope and natural wood, Percher Spider.
Crawl ball	1	For one week only throughout the experiment.	115 mm, with 3 × 58 mm holes, red-tinted polycarbonate.
Rope	1	For one week only throughout the experiment.	60 cm length of 1.5 cm diameter hemp rope.
Nylabone	1	For one week only throughout the experiment.	Regular size, original flavour, (36g), Nylabone.
Ladder	1	For one week only throughout the experiment.	9 step wooden ladder 35.5 cm.
Wood ball	1	For one week only throughout the experiment.	Roll 'N' chew (small), natural wood 9 cm diameter.

3. Behavioural observation:

All behavioural observations were carried out by the same experienced researcher throughout the study. The observer entered the experimental room at least five minutes before the scheduled start time of the observation to allow the rats to habituate to his presence (e.g. Hurst et al., 1999). Behaviour sampling, or conspicuous behaviour recording, was used to record each occurrence of a complete aggressive and defensive bout between dyads of rats in each experimental group. All aggressive bouts that ended up with a clear winner (dominant 'over' position) and loser (submissive 'on-back-posture' position) were recorded with the identity of the rat. A rat was identified as a winner when it ended the confrontation adopting the dominant over position (an animal stands with its forepaws or all paws over its opponent who adopts the submissive on-back-posture, lies on its back and exposes the ventral surface of the body) according to (Baenninger, 1967; Meaney and Stewart, 1981; Panksepp, 1981; Takahashi, 1986). This form of behaviour was chosen because it is easily measured (Panksepp, 1981), and represents the clear end point of aggressive encounters between rats in a laboratory situation.

Behaviour sampling was carried out, using a check sheet, in one session for the first hour of the dark phase (1200 hr to 1300 hr) for

three weeks throughout the experimental time. It started from the first week and continued every other week (1st, 3rd, and the 5th observation week) because other data were being collected from the rats in the other weeks (2nd, 4th and 6th week of the housing period).

4. Statistical analysis:

SPSS (version 12.0 for windows) was used for all statistical analyses. Data were checked for normality and homogeneity of variances to test for the suitability of using parametric tests. All data met assumptions of parametric statistics (normality, homogeneity of variance, linearity) (Fowler and Cohen, 1990).

Differences between the two housing systems in the frequency of successful aggressive and successful defensive bouts were tested using an independent *t*-test. Data are presented as estimated marginal means (EMM) \pm SE. For each housing system correlations between the total frequencies of successful aggressive and successful defensive bouts in each observation week and over the entire experimental period were measured using Pearson correlation test. To determine the form of social dominance of laboratory rat, a further correlation between successful aggressive and successful defensive bouts in each observation week and over the entire experimental period was done using a Partial correlation technique controlling for the effect of housing system and replicate.

RESULTS

1. Differences between housing systems:

As shown in figure 1, there was a significant effect to the housing system on the frequency of successful aggressive bouts. Rats housed in the unenriched systems were found to be more frequently involved in successful aggressive

bouts ($t_{10} = 4.46, P < 0.001$) as compared to those housed in the enriched systems. Similarly, as shown in figure 2, rats of unenriched systems were found to be more frequently involved in successful defensive bouts ($t_{10} = 3.82, P < 0.001$) as compared to those housed in the enriched systems.

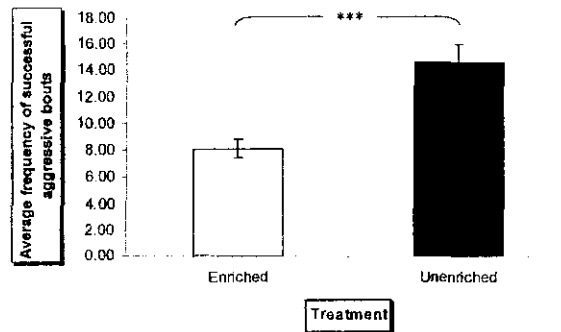


Figure 1: EMM \pm SE 'Average frequency of successful aggressive bouts' by the rats in the two housing systems. *** P < 0.001

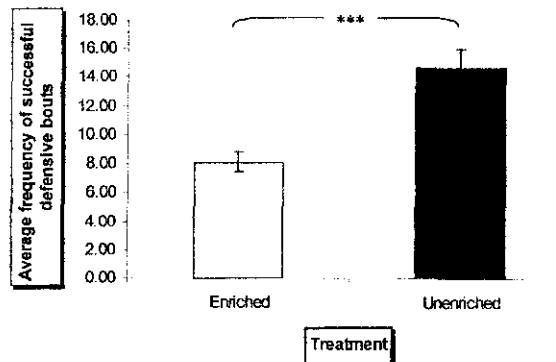


Figure 2: EMM \pm SE 'Average frequency of successful defensive bouts' by the rats in the two housing systems. *** P < 0.001

2. Effects of housing systems on dominance and its stability over time:

For the enriched housing system, both total frequency of successful aggressive bouts and successful defensive bouts in the first observation week correlated positively with those of the third observation week and with

the total frequency of successful aggressive and defensive bouts of the entire experimental period (Table 2 and 3). All of these findings were similarly evident when data of the unenriched housing system was analysed on its own.

Table 2: Pearson correlation coefficient of successful aggressive bouts over observation weeks and the entire experimental period.

	Ag1	Ag3	Ag5	AgT
Ag1	1	NS	0.32*	0.44*
Ag3	NS	1	0.48*	0.65**
Ag5	0.32*	0.48*	1	0.63**
AgT	0.44*	0.65**	0.63**	1

Ag= Successful aggressive bouts.

1, 2, 3 = 1st, 3rd and 5th observation week.

T= Entire experimental period.

* P < 0.05 ** P < 0.01

NS = non significant

Table 3: Pearson correlation coefficient of successful defensive bouts over observation weeks and the entire experimental period.

	Df1	Df3	Df5	DfT
Df1	1	NS	0.47*	0.49*
Df3	NS	1	0.44**	0.65**
Df5	0.47*	0.44**	1	0.62**
DfT	0.49*	0.65**	0.62**	1

Df= Successful defensive bouts.
 1, 2, 3 = 1st, 3rd and 5th observation week.
 T= Entire experimental period.
 * P <0.05 ** P <0.01
 NS = non significant

As shown in table 4 and 5, results of the partial correlation technique were also the same. Regardless the treatment and replicate, there were positive correlations between the total frequencies of both successful aggressive

and successful defensive bouts of the first observation week and those of the fifth observation week and the entire experimental period

Table 4: Partial correlation coefficient (*r*) of successful aggressive bouts over observation weeks and the entire experimental period.

	Ag1 Partial r	Ag3 Partial r	Ag5 Partial r	AgT Partial r
Ag1	1	NS	0.35*	0.67***
Ag3	NS	1	0.39**	0.50***
Ag5	0.35*	0.39**	1	0.51***
AgT	0.67***	0.50***	0.51***	1

Ag= Successful aggressive bouts.
 1, 2, 3 = 1st, 3rd and 5th observation week.
 T= Entire experimental period.
 * P <0.05 ** P <0.01 *** P <0.001
 NS = non significant

Table 5: Partial correlation coefficient (*r*) of successful defensive bouts over observation weeks and the entire experimental period.

	Df1	Df3	Df5	DfT
	Partial r	Partial r	Partial r	Partial r
Df1	1	NS	0.41*	0.66***
Df3	NS	1	0.44**	0.49***
Df5	0.41*	0.44**	1	0.71***
DfT	0.66***	0.49***	0.71***	1

Df= Successful defensive bouts.

1, 2, 3 = 1st, 3rd and 5th observation week.

T= Entire experimental period.

* P <0.05

** P <0.01

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DISCUSSION

The results of this experiment demonstrated that prolonged housing of rats in enriched cages greatly decreased their agonistic activities. One possibility is that in unenriched cages, due to the lack of any physical structures, rats themselves may be a more prominent focus of their cage-mates' attention, and therefore attract relatively more non-aggressive and aggressive interaction. Non-aggressive social investigation has previously been reported to promote aggressive responses (e.g. Robitaille and Bovet, 1976). The lack of cage structures may also simply increase the chance that two rats come into direct contact with each other.

Close proximity of animals was reported to increase aggression in mice (Van Loo et al., 2001), and the haphazard

collision of two running rats might also induce an aggressive response (e.g. Robitaille and Bovet, 1976). The provision of additional structures such as a shelter, bedding and nesting materials and crawling balls into enriched cages may also have provided rats with an increased opportunity to escape aggressive attacks by cage mates, whereas in unenriched cages, retreat may have been less easy to achieve, due to the lack of suitable shelters and refuges, resulting in a frustrated escape response (Hurst et al.,

1999). The ability to escape an attacker in enriched cages may have resulted in fewer decisive aggressive encounters whereas in unenriched cages, where escape may have been more difficult, loser rats may have ended up adopting the submissive posture more frequently, resulting in the dominant likely embarking in a bout of aggressive grooming (Hurst et al., 1999).

The reduction of agonistic behaviours in enriched conditions may also have been due to the nature of the husbandry procedures used in this experiment, cage cleaning in particular. Cages of all rats were cleaned out completely once a week. Whilst this process was carried out in exactly the same way for both housing types, it involved returning some of the enrichment items such as the shelter, uncleaned. The re-introduction of these items to the enriched cages may have imposed some level of familiarity to the environment (by providing familiar olfactory and visual cues), which may in turn have reduced agonistic interaction between rats in these cages. It has been shown in laboratory mice that incomplete cage cleaning regimes that allow retention of some uncleaned objects (e.g. nesting but not bedding materials) decreased inter-male aggression (Van Loo et al., 2000).

Orok-Edem and Key (1994) demonstrated that provision of some chewable items, such as gnawing blocks and wooden tongue depressors to the cages of

laboratory rats, reduced the frequency of fighting, although the study only lasted 5 days and any novelty effects may not have worn off in this time. It is possible that allowing the animals to exert simple control over the environment (e.g. by providing objects that can promote the expression of species-specific behaviours such as gnawing), motivation for agonistic interactions may decrease.

Reduced aggressive encounters and subsequent improvement in welfare, between members of group-housed animals in enriched conditions, have also been reported in pigs (O'Connell and Beattie, 1999), laying hens (Gvoryahu et al., 1994), captive primates (Kitchen and Martin, 1996) and mice (Armstrong et al., 1998; Van Loo et al., 2002, 2003). In contrast, other experiments have reported an increase in agonistic behaviours, particularly in enriched-housed mice (Haemisch and Gartner, 1997; Haemisch et al., 1994; Van Loo et al., 2002). The increase of agonistic behaviours in enriched cages was interpreted as to be due to either close proximity and exposure of parts of the body that are vulnerable for biting, such as tails (Van Loo et al., 2002), or stimulation of territorial behaviour (Haemisch and Gartner, 1997; Haemisch et al., 1994). This increased aggression in enriched environments appears to be a species difference in response to the housing environment, with male laboratory mice

Table 5: Partial correlation coefficient (*r*) of successful defensive bouts over observation weeks and the entire experimental period.

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Reduced aggressive encounters and subsequent improvement in welfare, between members of group-housed animals in enriched conditions, have also been reported in pigs (O'Connell and Beattie, 1999), laying hens (Gvoryahu et al., 1994), captive primates (Kitchen and Martin, 1996) and mice (Armstrong et al., 1998; Van Loo et al., 2002, 2003). In contrast, other experiments have reported an increase in agonistic behaviours, particularly in enriched-housed mice (Haemisch and Gartner, 1997; Haemisch et al., 1994; Van Loo et al., 2002). The increase of agonistic behaviours in enriched cages was interpreted as to be due to either close proximity and exposure of parts of the body that are vulnerable for biting, such as tails (Van Loo et al., 2002), or stimulation of territorial behaviour (Haemisch and Gartner, 1997; Haemisch et al., 1994). This increased aggression in enriched environments appears to be a species difference in response to the housing environment, with male laboratory mice

being less socially tolerant than other species (Gray and Hurst, 1995).

The results of the correlation test showed that, in enriched cages, there were significant positive correlations between the frequency of both successful aggressive and successful defensive bouts of rats over the observation weeks and between them and those of the total experimental period. The correlation between total frequencies of aggressive and defensive bouts of first and third week was not significant. These results were also evident for the rats in unenriched cages. This means that there was always a clear dominant and a clear subordinate animal in each cage of the enriched cages. It has previously been demonstrated that when laboratory rats are housed in groups in standard laboratory cages, they may behave aggressively towards each other in order to develop a stable social hierarchy and that it takes almost two to three weeks to develop (Stefanski, 2001; Stefanski et al., 2001). The results of this experiment demonstrate therefore that environmental enrichment, despite decreasing the agonistic interaction in laboratory cages, does not change dominance order. This could be due to the fact that the addition of physical items such as rodent retreat, wooden balls and perchers to cages may compartmentalize them into various partitions which may in turn allow animals to escape their conspecifics attacks and to mark their territories. The lack of positive

correlations between aggressive and defensive bouts of the first and third week may reflect that the dominance order was not yet formed and that it needs more than two weeks to develop.

The results of the partial correlation technique showed that there were also positive correlations between successful aggressive and successful defensive bouts over the observation weeks, except for the first and third week, and between them and those of the entire experimental period. This means that regardless, the housing system and replicate there was always a clear dominant and a clear subordinate animal within each cage. This finding is in accordance with those of Baenninger (1966) who reported that groups of rats (up to five animals per cage), if kept in harmony and undisturbed, can develop a stable form of nearly linear social hierarchy post weaning and over nine weeks time. The way that was used in this experiment to assign dominance score in laboratory rats appears therefore reliable under different housing environment of standard and enriched laboratory cages.

It can be concluded that environmental enrichment may affect some key behaviours of the laboratory rat such as agonistic interaction. Prolonged housing of rats at highly complex cages reduced their agonistic interaction and, most importantly, produced no change in the dominance order. Taking these findings together, it appears that

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.

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تأثيرات دعم المسكن علي السلوكيات العدوانية و الترتيب الاجتماعي في ذكور الجرذان المعملية

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تم إجراء هذه التجربة لدراسة تأثير دعم أقفاص التجارب وذلك بإضافة عناصر عديدة في الأقفاص على التفاعلات العدوانية والترتيب الاجتماعي لذكور الجرذان المعملية حديثة القطام. تم إسكان الجرذان المستخدمة في هذه التجربة وعددها ٧٢ في مجموعات مكونة من أربعة حيوانات في كل مجموعة في أقفاص إما 'عادية' أو 'مدعمة' لمدة ستة أسابيع والتي تم تكرارها ٣ مرات. تم تسجيل السلوكيات العدوانية، التي انتهت بفانز واضح وخاسر واضح، في الساعة الأولى من فترة الظلام لدورة الإضاءة في الأسبوع الأول والثالث والخامس خلال فترة الأسابيع الستة للتجربة. بينت النتائج أن الجرذان التي تم إسكانها في الأقفاص "المعددة البيئة" أظهرت انخفاض في مستويات نوبات السلوكيات العدوانية والدفاعية الناجحة بالمقارنة مع الجرذان التي تم إسكانها في أقفاص بيئتها "عادية". أظهرت النتائج أيضا أن دعم بيئة الأقفاص المعملية لجرذان التجارب لا يغير الترتيب الاجتماعي الخطي لتلك الحيوانات. وبالتالي، فإن دعم الأقفاص المعملية التقليدية يمكن أن يخفف من العجز السلوكي الذي يحفزها السكن لفترات طويلة في أقفاص عادية مثل إحباط الإستراتيجيات الإجتماعية وزيادة السلوكيات العدوانية وبالتالي يمكن أن يؤدي إلى تحسين مستويات الإراحة في جرذان التجارب.