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Removing individual rats affects indicators of welfare in the remaining group members

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Abstract

The removal of individuals from social groups, *e.g.* in order to maintain appropriate stocking densities in groups of rapidly growing young laboratory rats, is often necessary. However, such removals may be stressful and few studies have investigated their effects on the behaviour, physiology and welfare of the remaining group members. In this study we investigated this issue for rats housed at different stocking densities by observing behaviour and recording faecal corticosterone metabolite levels both before and after removal. We found that, irrespective of stocking density, the rats remaining in the home cage significantly increased agonistic behaviour, audible vocalization, aggressive grooming, bar-chewing and climbing behaviour following removal of their cage-mates, and that these behavioural changes were associated with a highly significant post-removal increase in their faecal corticosterone metabolite levels. Taking the behavioural and physiological results together, it appears that the removal of individuals from groups of young laboratory rats resulted in social stress, and thus an apparent impairment of welfare.

Keywords: Animal welfare; Laboratory rat; Social disruption; Social stress

1. Introduction

The social environment of a gregarious species such as the laboratory rat [1] is likely to have a major influence upon its welfare. Social isolation [2], over-crowding [3] and group composition [4] all appear able to affect behavioural and physiological indicators of welfare. It is therefore possible that standard husbandry procedures that disrupt the social environment, for instance through the disturbance of social odours (*e.g.* cage cleaning), the introduction of stressful procedures (*e.g.* handling) or *via* change to the composition of individuals within a cage, are also able to impact upon rat welfare [2]. Yet, whilst there has been some investigation into the potentially disruptive effect on the social environment, and thus welfare, of both cage cleaning [5] and handling [6,7], there has been little research into the effect of changing group composition on laboratory rat welfare.

A Council of Europe proposal to provide newly weaned rats with smaller space allowances when housed in larger cages will result in animals being raised at higher stocking densities in larger groups comprised of more litters [8]. In order to maintain the appropriate stocking densities in groups of rapidly growing weanling rats, such housing systems will inevitably require the removal of a proportion of individuals at particular time intervals. The removal of individuals from social groups will also frequently occur as young rats are shipped out to laboratories from breeding establishments, and when rats are removed for either experimental procedures or due to illness. During the removal process, individual rats are arbitrarily selected to be removed. We were therefore interested to see whether or not such removal – incorporating both the process of removal itself and the resulting change to group size – affects the social behaviour of the remaining rats, and whether it impacts on behavioural and physiological indicators of their welfare.

Previous research into the effect of changing group composition on laboratory rat welfare has focused on either

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membership rotation [9] or the introduction of new animals into a stable social group [10]. To our knowledge, the only study investigating removal effects on rodent social behaviour was carried out on mice housed in a population cage [11]. The authors found that the removal of several individuals (including dominant males) resulted in an increase in the levels of agonism amongst the remaining animals, as previously subordinate mice attempted to establish territories. However, it is unclear whether we might expect similar results for rats, particularly for young rats in single sex groups housed in standard laboratory cages, and whether the welfare of those animals that remain in the group would be affected. Research on other species has investigated the effect of removing individuals on the behaviour of the remaining members of the group, either finding no subsequent behavioural change [pigs: [12]] or destabilisation of the social system following the removal of key individuals (*e.g.* dominant males) [primates: [13–15]].

Therefore, the aim of this experiment was to investigate the effect of removing individuals from groups of young laboratory rats on behaviour, physiology and thus the welfare of the remaining animals. Rats were studied at different group sizes and stocking densities, as part of a larger project and so as to investigate whether these factors influenced response to removal.

2. Method

2.1. General animal housing and husbandry

This study was carried out over a period of 12 months in six separate replicates as part of a large project investigating the long-term effects of stocking density on multiple measures of welfare in laboratory rats. The subject animals were male newly weaned rats, 35–49 g weight at arrival, of the Wistar Hannover (outbred) strain (Harlan, Bicester, UK). We used a total of 348 rats divided between six replicates, with each replicate of 58 rats derived from 13 different litters. For each replicate, the rats were housed in the same room throughout the study on a 12 hour light/dark cycle (lights on 0200–1400) with continuous dim red light (60 W, 380 lm) enabling observation during the dark period. The room was maintained at a constant temperature (20 ± 1 °C) and humidity (46% relative humidity). Food (Harlan Teklad Laboratory Diet) and water were provided *ad libitum*. Cages contained sawdust litter and shredded paper, were checked daily and were cleaned out on a weekly basis the day before removal took place.

2.2. Arrival

For each replicate, upon arrival the 58 rats were housed in four cages (70 × 50 × 35 cm) in arbitrarily selected groups. The dimensions of these initial cages differed to those of the treatment cages so that prior to the start of the experiment all the rats were unfamiliar with the dimensions of the particular cage size treatments to which they were to be allocated. We arbitrarily selected 24 rats to act as focal rats for the course of the whole replicate. These 24 focal rats were dye-marked (Clairol Nice n'easy Natural Black) [2] to allow individual identification, and their marks were refreshed mid-way through the experiment. On both occasions there was at least 48 h after dye-marking before the next behavioural observation in order to minimise any potential effect of dye-marking on behaviour. The focal rats also had identification marks added on to their tails using a permanent marker pen (Pentel), and these marks were refreshed every week following cage cleaning. The rats remained housed in the initial 'arrival' groups for at least 5 days to allow them to acclimatise to the lighting regime and new environment.

2.3. Experimental procedures

2.3.1. Treatments

Following acclimatisation, focal (marked) and non-focal (unmarked) rats were arbitrarily allocated to one of three treatments based on two different cage sizes either 1600 cm^2 ('small') or 2500 cm^2 ('large'), and two different stocking densities, based on proposals from the Council of Europe. The three treatments were: 'small/low' (small cage (1600 cm^2)/low stocking density); 'large/low' (large cage (2500 cm^2)/low stocking density); 'large/high' (large cage (2500 cm^2)/high stocking density) (see Fig. 1 for details (including stocking densities)). All three housing treatments had flat wire lids 18 cm high. Council of Europe proposals (Council of Europe, 2000; Francis, 2000) suggest that stocking densities of weanling rats at breeding establishments can be increased if they are housed in larger cages (*e.g.* 2500 cm^2 or greater). The small/low and large/high treatments therefore allowed us to investigate the effect of these housing procedures. The decision to include the additional large/low treatment allowed us to make further comparisons between the treatments. We did not include a fourth treatment (that would have completed a 2 × 2 design) because of practical/time constraints.

Each of the six replicates were carried out separately, and consisted of one example of each of the three housing treatments. Thus, after the completion of the six replicates, we had collected data from six examples of each treatment ($n = 6$), a total of 18 cages ($N = 18$). Within each replicate the housing treatments were allocated a group number (1–3), and this group number dictated the order in which the various procedures, such as cage cleaning and behavioural observations *etc.*, were carried out. The allocation of group number was balanced between the housing treatments such that any effect of group number was the same for all treatments. The group number also dictated the position of each cage within the study room. All three cages were kept on tables to allow access for behavioural observation, and were separated from one another by non-transparent plastic barriers (75 cm × 75 cm). These were intended to prevent visual contact between the cages, may also have limited the transmission of some auditory and olfactory cues, and minimised disturbance from the movement of researchers in the study room.

2.3.2. Mixing

The experimental animals were mixed into their allocated treatment at 1000 during the 'lights on' phase of the light cycle as would be done at a breeding establishment, staggered over a three day period (one cage/day) balanced for treatment. Eight focal rats and the required number of non-focal (unmarked) rats were arbitrarily allocated to each treatment.