The effects of cage cleaning on aggression within groups of male laboratory mice

SAMANTHA GRAY & JANE L. HURST

Behaviour and Ecology Research Group, Department of Life Science, University of Nottingham, University Park, Nottingham NG7 2RD, U.K.

(Received 15 November 1993; initial acceptance 11 February 1994; final acceptance 23 May 1994; MS number: 4529)

Abstract. Cage cleaning is an unavoidable but frequent source of disturbance for mice maintained in the laboratory. A series of tests was conducted to assess how physical disturbance, the removal of odour cues and exposure to the odours of strangers experienced during cage cleaning affects aggression within established groups of male CFLP mice, Mus musculus. Handling mice decreased the latency to attack and increased the frequency and duration of aggression within groups. Transferring mice into completely clean cages reduced aggression compared to those replaced in home cages that had not been cleaned. Exposure to the odours of strangers while males were held temporarily in a handling bin had no significant effect on aggression when the males were transferred to clean cages or returned to their home cages. Aggression was greatest when mice were replaced in home cages that had not been cleaned but that had a clean sawdust substrate, and decreased with increasing removal of home cage odours on the cage base and grill. Common cage-cleaning practices in which only the substrate and parts of the cage are cleaned, and other procedures that involve daytime handling and replacing mice in their home cage may thus promote aggression within male groups. Transferring mice into completely clean cages is recommended when aggression within caged groups of males is a concern.

Aggression within caged groups of male mice can be a serious welfare problem for maintaining mice in the laboratory, regardless of any additional stress inflicted by experimental procedures. Given the large number of mice used in scientific research, living conditions and maintenance procedures designed to minimize this problem could have a major impact on the stress experienced daily by mice forced into unnaturally close confinement. Aggression between mice is mediated by social odour cues emanating from the body and deposited on the substrate, originating largely from their urine. These cues are involved both in the advertisement of dominance over a defended territory (Gosling & McKay 1990; Hurst 1990, 1993) and in the recognition and maintenance of social tolerance between familiar individuals (Hurst et al. 1993). Cage cleaning, while essential for hygiene and to prevent the build-up of excreta, disrupts these odour cues and stimulates activity especially during the daytime when mice are normally inactive. Additionally, mice may be exposed to foreign odours during handling. All of these factors could have a significant effect on aggression within caged groups (e.g. Poole 1987) although the effects of different maintenance procedures and regimes on aggression appear to be unknown. Cleaning may be complete or partial (e.g. only substrate refreshed) and of variable frequency, but is usually carried out at least once or twice per week.

Experiments designed to provide an understanding of the way in which mice use odour cues lead to differing expectations concerning the likely effects of cage cleaning on aggression between familiar males. First, dominant male mice deposit urine cues around their territory to advertise their dominance over the area, and use the presence of their own odour to recognize the area that they defend. Highly aggressive dominant males are thus much more aggressive towards unfamiliar males (Jones & Nowell 1973, 1975) and familiar subordinates (Hurst 1990, 1993) when surrounded by their own odour cues. This leads to the prediction that removing territorial odour cues during cleaning will reduce the aggression of dominant males. However, aggression within male groups also declines as mice become highly familiar and social relationships become well established (Poole & Morgan 1973). Removing odour cues
that signal the clear dominance of one male might thus decrease social stability by increasing challenges from subordinate males with a concomitant response from the dominant. Furthermore, recent findings have shown that the odours of subordinates on the substrate are important in maintaining tolerance between familiar males (Hurst et al. 1993), predicting that the removal of substrate odours will increase aggression against subordinates by both dominants and fellow subordinates.

We thus set out to investigate how routine cage cleaning and the disturbance induced by handling affect aggression in caged groups of male laboratory mice. Since common practices involve different degrees of cleaning, from simply replacing soiled substrate with clean to transferring mice into completely clean cages, we tested the effects of disturbing different aspects of their olfactory environment. We also investigated the effects of providing mice with an object to mark that could easily be transferred between cages to maintain established marks within their environment. Furthermore, since exposure to the odours of unfamiliar males can stimulate aggression (Mugford 1973; Hurst 1993), we also investigated whether the use of handling bins soiled by many other males, commonly used during cleaning and other procedures, potentiated aggression between familiar males.

METHODS

The subjects of this study were 50 male mice of the CFLP strain, obtained at an age of 80 days from Bantin and Kingman Laboratories, Hull, U.K. On arrival they were housed as 10 groups of five in polypropylene cages (44 × 26 × 12 cm) on sawdust substrate (Fig. 1). All cages were housed in the same support rack. They were maintained on ad libitum food (41-B Modified 441, Pilsbury, U.K.) and water throughout the study. All mice were kept on a 12:12 h light:dark cycle, with white lights on at 0715 hours. The temperature range in the animal unit was 21–22°C. Mice within each group were given a unique identifying mark using Clairol black hair dye. After arrival the mice were cleaned out normally as outlined below. No observations were made for the first 10 days, allowing the mice to acclimatize to the new surroundings and establish their social relationships.

The normal weekly cleaning routine in our Animal Unit was as follows: Monday: sawdust substrate scraped out and replaced with clean; Wednesday: water bottles exchanged for clean; Thursday: entire cage, except for water bottle, exchanged for clean. During cleaning, a cage was removed from the rack and the mice placed in a bucket while the substrate/cage was exchanged. The mice were then tipped back into the clean or partially clean cage which was replaced in the rack. Cleaning always took place during the light period.

Prior to the experimental treatments aggression was scored in all 10 groups over a period of 5 h following both a complete cage clean and when mice were replaced back into their own dirty cage to assess the time course of aggression following cage cleaning and to identify the dominant male within each group. Since aggression rose immediately after cage cleaning and remained high for approximately 15 min, observation periods of 20 min immediately after a cleaning treatment were used in the experiment. The dominant male in each group was identified as the individual that initiated most attacks. The identity of the dominant male changed in seven of the 10 cages during the study.

The main study was performed during the light phase of the light:dark cycle, between 0900 and 1400 hours, since cage cleaning is usually carried out during the light phase in most animal facilities. Ten groups of mice were each subjected to 10 different treatments, as described in Table I. These treatments were given in random order to each group, except that no two consecutive treatments could involve mice being replaced in a cage containing soiled substrate, as this was considered too unhygienic. The odours on cage bases, grills and marking blocks that were not cleaned were thus deposited over a period of 3–7 days. Directly after treatment the group was observed for 20 min and the behaviour of each individual dictated onto
Table 1. Experimental treatments

<table>
<thead>
<tr>
<th>Handling bin</th>
<th>Cage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 —</td>
<td>Minimal disturbance control: cage removed from rack and food pellets from hopper for observation</td>
</tr>
<tr>
<td>1 Clean</td>
<td>Uncleaned and undisturbed home cage and substrate</td>
</tr>
<tr>
<td>2 Clean</td>
<td>Completely clean cage and substrate</td>
</tr>
<tr>
<td>3 Soiled</td>
<td>Uncleaned home cage, soiled substrate removed and replaced</td>
</tr>
<tr>
<td>4 Soiled</td>
<td>Uncleaned cage base and grill top: clean substrate and marking block</td>
</tr>
<tr>
<td>5 Clean</td>
<td>Uncleaned cage base; clean grill top, substrate and marking block</td>
</tr>
<tr>
<td>6 Clean</td>
<td>Completely clean cage and substrate: soiled marking block transferred from home cage</td>
</tr>
</tbody>
</table>

audio cassette, and later transcribed using the 'Observer' computer software to calculate the duration and frequencies of behavioural elements performed by each mouse (Noldus Ltd, Wageningen, the Netherlands). Behaviour was divided into three categories for analysis: non-social cage investigation, social investigation and aggression. All five mice were observed for social-investigative and aggressive behaviour, and the identity of both the actor and the receiver was dictated onto audio cassette. Social investigation was divided into two elements: 'sniff', defined as contact between the nose of the actor and the body of the receiver (excluding the anogenital region), and 'ano' when the nose of the actor was in contact with the anogenital region of the receiver. The aggression category included attacking, chasing, threatening, biting and fighting (see Mackintosh 1981). Non-social investigation was recorded for only the dominant male and one subordinate (selected at random), together with the part of the cage investigated (see Fig. 1), as it was impossible to observe all mice simultaneously for this category of behaviour.

Ethical Note

We should point out that the aggression measured in this study was a response to normal husbandry procedures and thus is likely to reflect that experienced regularly by caged groups of male mice. The CFLP strain was chosen since they show intermediate levels of aggression compared with other strains which typically show very high or low levels.

Data Analysis

Non-social investigation was analysed using a Multifactor ANOVA, as the data closely approximated a normal distribution. Social investigation was similarly analysed using a Multifactor ANOVA, after logarithmic transformation provided a normal distribution. Since the level of aggression was highly variable between the 10 cages used in the study, the measures of aggression (both duration and frequency totalled per group) were ranked within groups to show their relative response to the different treatments and analysed by Meddis (1984) tests (a non-parametric equivalent of analysis of variance). Aggressive behaviour was analysed as the total duration and frequency within each group since individual aggression was not independent, and the data examined subsequently to assess whether responses were due to the dominant or subordinate individuals or both.

RESULTS

Effects of Physical Disturbance

We predicted that the physical disturbance involved in cage cleaning would lead to increased aggression through increasing the alertness and activity of mice during the daytime. To examine the effects of physical disturbance we compared behaviour in treatments 0 (cage disturbed but mice not handled), 1 (mice handled and replaced in their undisturbed home cage) and 5 (as treatment 1 but with physical disturbance of the home cage substrate). Handling the mice and replacing them in their home cage reduced the latency to the first attack after treatment ($z=2.15$, $P<0.05$) and increased the total duration of aggression observed in each group (Fig. 2; $z=2.76$, $P<0.01$), and the mean duration of each aggressive interaction ($z=2.76$, $P<0.01$). This effect was apparent among both dominant and subordinate males. Disturbing the substrate appeared to have no significant effect on their relative aggressive response (Fig. 2).
Treatment

Figure 2. Comparative duration of aggression in response to increasing levels of physical disturbance ($X \pm SE$ rank of the total duration of aggression within groups). The duration of aggression in response to the three treatments was ranked 1–3 within each group to take differences in aggressiveness into account. 0: Cage disturbed (actual duration of aggression 81.41 ± 48.48 s); 1: cage disturbed, mice handled (87.46 ± 34.04 s); 5: as 1 plus substrate disturbed (70.55 ± 31.22 s).

Removal of Familiar Odours and Exposure to Unfamiliar Odours

A two-factor Meddis test compared the effects of removing all familiar odour cues (treatments 2, 4) versus leaving home cage odours undisturbed (treatments 1, 3) and exposure to a clean handling bin (treatments 1, 2) or one bearing the odours of unfamiliar males (treatments 3, 4) on subsequent aggression. Since it was not clear whether cage odours would increase or decrease aggression among some or all mice, general tests for any significant differences were carried out. Mice replaced in uncleaned home cages were significantly more aggressive than those transferred to clean cages (Fig. 3: $H=3.79$, $P<0.05$), with a shorter latency to the first attack ($H=4.59$, $P<0.05$). Examining behaviour between different dyads, the total duration and frequency of aggression, and the duration of each aggressive encounter, were elevated significantly in interactions between two subordinates only ($P<0.01$). Exposure to the odours of foreign males in the handling bin had no significant effects on aggression (Fig. 3).

The reduced aggression observed in clean cages was not due to mice spending their time investigating the clean cage instead of interacting with one another. While the frequency of cage investigation was much higher in clean cages ($F_{1,74}=67.3$, $P<0.001$) there was no difference in the total duration of investigation ($F_{1,74}=0.02$, ns) since the duration per investigatory bout was much less than in soiled cages ($F_{1,74}=24.6$, $P<0.001$). The presence of home cage odours induced a significant increase in both the total duration ($F_{1,37}=9.96$, $P<0.01$) and frequency ($F_{1,37}=4.37$, $P<0.05$) of social investigation per group, especially between subordinate males.

Effect of Increasing Odour Removal

Since mice placed in completely clean cages were less aggressive than those replaced in uncleaned home cages, we tested whether aggression decreased as an increasing proportion of home cage odours were removed. As predicted, males spent less time in aggressive behaviour when more of their home cage odours were removed (Fig. 4: $z=2.81$, $P<0.01$). This was due to both a decrease in the frequency of agonistic behaviour ($z=1.97$, $P<0.05$) and a reduction in the mean duration of each aggressive interaction ($z=2.41$, $P<0.01$). Providing mice with a clean top grill and water bottle (treatments 8, 9, 2) resulted in the greatest reduction in aggression compared with behaviour in their uncleaned home cage.
Treatment 5: Physical disturbance of substrate (actual duration of aggression 70.55 ± 31.22 s); 6: substrate and marking block clean (108.5 ± 39.52 s); 7: cage base, substrate and marking block clean (59.36 ± 21.49 s); 8: grill top, substrate and marking block clean (42.62 ± 22.38 s); 9: whole cage clean, soiled marking block transferred (81.41 ± 48.48 s); 2: entire cage clean (52.7 ± 27.47 s).

(treatment 5) while retaining the soiled home cage grill but cleaning the cage base (treatment 7) had an intermediate effect (Fig. 4). However, replacing soiled substrate with clean did not reduce aggression. To the contrary, placing clean sawdust in a soiled home cage (treatment 6) stimulated more aggression from the dominant male (those responsible for the most aggression within groups) than any other treatment (Fig. 4). Latencies to aggression showed a corresponding difference between treatments, increasing with increasing removal of home cage odour cues. The decrease in total aggression with the removal of home cage odours was not just due to the increasing delay to first attack, however. The rate of aggression over the observation period remaining after the first attack also decreased with increasing removal of home cage odour cues (z=2.61, P<0.01).

DISCUSSION

Our results show that male mice were more aggressive in their own home territory, where they were surrounded by home cage odours, than when in a clean cage, in accordance with findings by Jones & Nowell (1973, 1975). Removing home cage odours did not appear to increase challenges from subordinate males for dominance over an apparently unoccupied area. This may have been due to the familiarity between the males since social relationships should have been well established. While two highly aggressive males might fight for dominance over clean, unoccupied areas, subordinate males are unlikely to challenge a familiar dominant competitor, regardless of the surrounding olfactory environment, because their experience should allow them to recognize the greater competitive ability of this male. Complete cage cleaning thus does not potentiate aggression above that provoked by simple disturbance during the daytime.

Our findings have important implications for the common practice of partial cage cleaning in which only the substrate is cleaned, as this procedure appears to stimulate the greatest levels of aggression within groups of familiar adult male mice. Under these conditions mice are surrounded by their own home territory odours on the cage grill and base, which stimulates resident aggression (Jones & Nowell 1973). Group members whose odours do not match those on the clean substrate further stimulate attack because dominant males use substrate odours to discriminate between familiar subordinates currently resident in their territory and those that have dispersed (Hurst et al. 1993). This suggests that the distribution of odour cues used to recognize home territory by the dominant male might differ from that used to recognize group members. In wild populations, all group members deposit a low density of marks on any unmarked substrate in their home area, while concentrated marks deposited to advertise the identity of the dominant territorial male often form raised marking posts in particular sites (Hurst 1987, 1993). In our cages, odours on the grill top were particularly important in stimulating aggression, probably because the grill had a deep trough to carry a water bottle and food pellets, behind which the mice sheltered. They thus had extensive physical and olfactory contact with the grill whether resting or active.

Jones & Nowell (1973) suggested that greater aggression among mice introduced to environments containing familiar olfactory cues is due to competing types of behaviour, such as
investigation, being exhibited when mice are introduced into an unfamiliar environment. However, our results show that social investigation and aggression were both significantly higher in dirty cages while there was no difference in the amount of time spent investigating a clean cage. Thus mice replaced in clean cages showed an overall reduction in activity.

While it may be considered desirable to provide animals with a familiar 'natural' olfactory environment, this appears to lead to increased social pressure when male mice are closely confined together in captivity. The potentiation of aggression by home cage odours after mice were disturbed by handling further suggests that other procedures in which mice are removed temporarily from their home cage during the daytime will stimulate high levels of aggression when they are replaced back into their uncleaned home cage. We would thus recommend that mice are transferred into completely clean cages when aggression within caged groups of males is a concern. This is particularly important for procedures such as cage cleaning which involve frequent daytime disturbance, adding cumulatively to the attacks experienced by subordinate males.

ACKNOWLEDGMENTS

Many thanks to the staff of the Animal Unit for their advice and patience throughout this study. J.L.H. was supported by an Advanced Fellowship from the Science and Engineering Research Council.

REFERENCES


