

## Acute effects of cage cleaning at different frequencies on laboratory rat behaviour and welfare

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### Abstract

In rodents, cage cleaning increases cardiovascular and behavioural activity for several hours, which are commonly interpreted as stress responses. In mice, post-cleaning activity also includes aggression, which can cause serious injuries. This study was part of a long-term investigation into the effects of cage cleaning frequency on rat behaviour and welfare. Here we aimed to ascertain whether post-cleaning activity is stress- or aggression-related, thereby leading to recurrent acute reductions in welfare, or simply a result of non-aversive stimulation. Male Wistar ( $n = 160$ ) and Sprague–Dawley ( $n = 160$ ) rats, kept in four animal units, had their cages cleaned twice per week, once per week or once per fortnight, and were kept on one of two types of bedding. Behaviours were recorded in detail before and after cage cleaning for 20 weeks, as was the aversion-related Harderian gland secretion, chromodacryorrhoea ('red tears'). Cage cleaning caused decreased resting and increased feeding, walking, bedding manipulation and sheltering for at least 30 min after the disturbance. Skirmishing also increased markedly for 15 min after cleaning, but decreased thereafter to below baseline levels. Unlike in mice, all skirmishing was non-injurious and play-like. The frequency of cage cleaning did not affect the magnitude of this skirmishing peak, but rats that had their cages cleaned more frequently settled more quickly after cleaning. Surprisingly, chromodacryorrhoea decreased after cage cleaning; this could mean that rats find soiled cages stressful or alternatively, like many disturbances, cage cleaning might provoke frequent, curtailed bouts of grooming, thereby removing the secretion. Rats also manipulated aspen bedding more than paper bedding. Overall, we found no evidence that cage cleaning caused rats any acute decrease in welfare — a finding consistent with additional data we have obtained on the lack of preference by rats for soiled over clean cages, and a lack of long-term, behavioural and physiological responses to being cleaned frequently or infrequently.

**Keywords:** aggression, animal welfare, behaviour, husbandry, rats, stress

### Introduction

The cages of laboratory rats are routinely cleaned between three times per week and once every two weeks (fortnightly) depending on the animal facility. The frequency of cage cleaning is likely to affect how habituated the rats become to the cleaning process; therefore, the acute behavioural and physiological responses of rats to the cleaning process may differ. In addition, the frequency of cleaning affects how soiled the rats' cages become, influencing, for example, the concentrations of pollutants (Broderson *et al* 1976; Perkins & Lipman 1995; Ishii *et al* 1998; Carissimi *et al* 2000), micro-organisms (Borrello *et al* 1998), and scent signals, which may in turn affect the rats' behaviour and physiology even when 'undisturbed'. Cage cleaning is likely to be a significant event in the lives of animals that are otherwise rarely handled, and which live in environments with relatively little stimulation — as is currently the case for most laboratory animals.

Researchers seldom take into account when their animals' cages are cleaned when planning experiments, and indeed may not even be aware of when these events take place. Cage cleanliness can directly affect the performances of animals in behavioural tests. For example, gerbils were less able to discriminate odours in a novel arena on days when their cages were soiled compared with days when their cages had just been cleaned (Dagg *et al* 1971). Also, rats that were trained to anticipate 'tickling' by a human in an arena, emitted fewer anticipatory ultrasonic chirps when tested on the day of cage cleaning than on other days (Panksepp & Burgdorf 2000). More generally, cage cleaning has been shown to increase blood pressure, heart rate, movement, rearing behaviour and grooming in rats for at least 45–60 min after being returned to the cages (Duke *et al* 2001). Furthermore, Sharp *et al* (2002b) found that increased cardiovascular activity was still detectable after 3 h and was more pronounced in individually housed rats than in group housed rats. In another study,

Saibaba *et al* (1996) found that defaecation, and behaviours associated with exploration and movement were higher on both the mornings and afternoons of cleaning days compared with the equivalent time periods on non-cleaning days. Therefore, cage cleaning is thought to have a considerable impact on responses that potentially indicate acute stress in rats. Indeed, a study that compared the effects of different stressors in rats found that general activity, heart rate and blood pressure were elevated above baseline levels for 2 h after cage cleaning, whereas injections of saline caused increases for only 1 h (Schnecko *et al* 1998). Even indirectly witnessing the cages of other rats being cleaned significantly increased the heart rates of rats within the same room (Sharp *et al* 2003).

These responses are heightened by the fact that cage cleaning usually occurs during the light period, which allows the technicians good visibility for inspection of the animals but disturbs nocturnal and crepuscular animals — including rats and mice — during their resting period. Increases in general activity, heart rate and blood pressure in response to a variety of stressors, including cage cleaning, are more pronounced in rats if the stressor occurs during the light period than during the dark period (Schnecko *et al* 1998). Indeed, Doerning (1999) found that for more than 2 h after cage cleaning the body temperatures, blood pressures, activity levels and heart rates of rats were elevated to levels similar to those observed during their most active phase. Similarly in hamsters and gerbils, physiological responses to cage cleaning were stronger than for resident-intruder confrontations, vaginal smears or handling, and were more pronounced if the stressor occurred during the light period than during the dark period (Gattermann & Weinandy 1996; Weinandy & Gattermann 1997).

Although this evidence of increased cardiovascular and general activity post-cleaning suggests that rats find cage cleaning to be a stressful event — and therefore cage cleaning is often described as a ‘stressor’ (Schnecko *et al* 1998; Sharp *et al* 2002a; Sharp *et al* 2002b) — it is not in fact clear whether the stimulation associated with cage cleaning is aversive, neutral or even positive for the rats. In humans, heart rate, blood pressure and activity levels, together with sympathetic-adrenal activity, occurs in situations when subjects report feelings of fear and when they report feelings of arousal or excitement (eg Toates 1995). Therefore, cardiovascular measurements and general activity can be ambiguous indicators of welfare because they measure arousal but not necessarily valence (pleasantness or unpleasantness). Although there is no obvious infallible welfare indicator, this study monitored chromodacryorrhoea (‘red tears’ or ‘bloody tears’), which does not appear to be directly influenced by sympathetic activity (Harkness & Ridgway 1980).

Chromodacryorrhoea is a dark red stress-related secretion produced by the Harderian gland behind the eye, and which appears around the eyes and/or noses of rats. It is produced in response to a variety of stressors including restraint

(Harkness & Ridgway 1980; Singh *et al* 2001), temporomandibular joint pain (Kerins *et al* 2003), morphine withdrawal (Hepburn *et al* 1997; Rohde & Basbaum 1998), bright light (Hugo *et al* 1987), lack of in-cage enrichment (Foulkes 2004) and even mild disturbances (Mason *et al* 2004). It is related to the parasympathetic stress response (Harkness & Ridgway 1980; Clement 1994), and appears to have one major advantage over many other measures of stress in that it has never yet been reported to increase as a result of physical activity *per se* or ‘excitement’. Increases in chromodacryorrhoea can be visible within a few minutes of a stressor and can persist for several hours, depending on the nature of the stressor and the age of the rat (Harkness & Ridgway 1980; Singh *et al* 2001; Mason *et al* 2004). If cage cleaning is aversive, rather than neutrally or positively stimulating, it would therefore be expected for chromodacryorrhoea to be higher after cage cleaning than before it.

We also monitored and attempted to characterise the relative aggression levels of rats that experienced cage cleaning at different frequencies. In groups of male mice, aggression can be a serious problem and it has been shown to significantly increase for approximately 15 min after cage cleaning (Gray & Hurst 1995; Van Loo *et al* 2000). However, none of the previously cited studies involving socially housed rats stated whether the increased ‘activity’ levels observed after cage cleaning also included aggression, and if so, whether the aggression was injurious or ‘playful’ (Pellis & Pellis 1987); therefore, the current study also aimed to determine the nature of the aggressive behaviour. To avoid teleological language, we have used the term ‘skirmishing’ to describe the suite of behaviours that are often assumed to be aggressive in rats. Rats are known to continue play-fighting with familiar individuals well beyond puberty (Adams & Boice 1983; Takahashi & Lore 1983; Pellis & Pellis 1992; Smith *et al* 1999), although as adults they are obviously also capable of injurious fighting (Adams & Boice 1983; Smith *et al* 1999).

The results presented in this paper are part of a long-term study into the effects of different cage cleaning frequencies and bedding types on the health and welfare of rats (Burn *et al* 2006 *in press*). However, here we concentrate on the acute effects of cage cleaning on the behaviour of rats, which, if ignored in behavioural or physiological studies could potentially influence the experimental results. We wondered whether the frequency of cage cleaning would affect the degree of acute responses, the behaviours provoked, and how long it would take for the rats to return to ‘baseline’ (day before cleaning) levels of resting. For example, the rats housed in cages that were cleaned once per fortnight would have the dirtiest cages before cleaning, and would perhaps also be less habituated to the cleaning process, having experienced it only 10 times over the 20 weeks of the experiment, compared with the 40 times experienced by the rats housed in cages that were cleaned twice per week. Such factors may well affect how rats react to each individual cage cleaning experience.

Male rats of the two most commonly used stocks in the UK were chosen and the experiment was repeated in four university animal units. Although within-cage parameters were standardised as much as practically possible across the animal units, the units themselves were not specifically standardised against each other because they should, to some extent, represent the degree of standardisation between animal units in the UK generally. The details of the animal unit effects on results are presented in Burn *et al* (2006 in press). Our aim was to verify that any effects of cage cleaning on rat behaviour and physiology would be applicable across similar systems, so all the results here are those that were consistent across the different animal units.

## Materials and methods

### Animals and housing

The subjects were 160 Wistar and 160 Sprague–Dawley male rats (Harlan, Bicester, UK). The rats weighed 50–70 g at the start of the experiment and had just been weaned. They were housed in single-stock groups of four individuals in polyethylene cages (45–50 × 32 × 20–25 cm, length × width × height), each of which contained a paper ‘Des–Res’ shelter and a wooden chewing block (Lillico, Surrey, UK). The 80 cages were allocated to four different conventional animal units (Units A, B, C and D) within the University in a balanced design, such that each animal unit contained 20 cages. The cages were randomised within the racks with respect to treatment group and the cage positions were rotated every 2 weeks after observations were completed; the experiment ran for 20 weeks.

Environmental parameters varied between animal units but were within UK Home Office limits (Home Office 1995). Specifically, across the four animal units the temperature was 19–23°C, humidity was 40–65%, and there were 15–25 air changes h<sup>-1</sup> for ventilation. However, Home Office limits might sometimes have been exceeded in Unit D because of building work. Light:dark cycles were 12 h:12 h in Unit A (0700h–1900h) and Unit C (0800h–2000h) and 14 h:10 h in Unit B (0600h–2000h) and Unit D (0500h–1900h).

All rats were provided with water *ad libitum* and with each animal unit’s normal pelleted rat chow: Unit A: RM1 (E) pelleted diet (Special Diet Services, Witham, UK); Unit B and Unit C: RM3 pelleted diet (Special Diet Services, Witham, UK); and Unit D: Harlan Teklad FFG (Harlan Teklad, Bicester, UK). Rats in Unit A and Unit C were also provided with seed mixtures in the bedding each week (forage mix and mixed corn: Lillico, Surrey, UK); in addition, rats in Unit A were provided with peanuts.

Cage cleaning involved the replacement of the cage body and all the bedding; Unit A and Unit C retained the cage lid, whereas Unit B and Unit D did not. The technicians generally did not wear latex gloves when handling the rats during cage cleaning. The shelters and chewing blocks were replaced every two weeks, when all the cages were cleaned, but it was also necessary to replace the shelters more frequently than this because most rats destroyed them within a few days.

The experiment was approved by the University’s ethical review process, and statistical power tests were used to determine the appropriate sample size before the study began.

### Treatments

Cages were cleaned at three different frequencies: twice per week, once per week or once per fortnight, between 0830h and 1030h, at a regular time pre-arranged with each animal unit. Bedding was either aspen chip bedding (Units A, B and D: grade 8 [Lillico, Surrey, UK]; Unit C: QC bedding [B&K Universal Ltd, Hull, UK]) absorbent paper bedding (Alpha–Dri™ [Lillico, Surrey, UK], or occasionally Omega–Dri™ [Harlan Teklad]). The treatments were as follows:

- (1) Cleaned twice per week with aspen chip bedding;
- (2) Cleaned twice per week with paper bedding;
- (3) Cleaned once per week with aspen chip bedding;
- (4) Cleaned once per week with paper bedding;
- (5) Cleaned once per fortnight with paper bedding.

Aspen chip bedding was not used with the fortnightly cleaning frequency because previous studies had indicated that the ammonia concentration might reach levels that were unsafe for the animals and the humans involved (Broderson *et al* 1976; Perkins & Lipman 1995; Ishii *et al* 1998; Carissimi *et al* 2000). Technicians were instructed to fill all cages with bedding to a depth of 2 cm.

### Behavioural observations

Formal recording of behaviour started during week 6 of the experiment, when the rats were 9–10 weeks old, and continued for the duration of the experiment (ie for 14 weeks). Behaviour was observed every two weeks both on the day before all cages were cleaned and on the day that cleaning took place. These observations were made between 0830h and 1030h on both days, depending on when cage cleaning had been agreed to take place, and consisted of three instantaneous recordings of each cage. The observations were made immediately after cage cleaning (as soon as possible after the cage was placed back in the rack), and then 15 min and 30 min afterwards, and also at the corresponding three time points on the day before. The behaviours recorded are listed in Table 1.

Individual rats could not be distinguished from each other so each behaviour was recorded as the number of rats performing that behaviour per cage. In addition, selected rare behaviours, including allogrooming, skirmishing, and squeaking, were noted whenever they occurred during the periods 0–15, 16–30 and 31–45 min after cleaning, and during the same time periods on the day before cleaning. Apart from allogrooming, these behaviours could usually be easily recorded because they were accompanied by sound.

The data were recorded live so the observer’s presence may have influenced the rats’ behaviour. However, observations were usually made while technicians carried out routine husbandry procedures in close proximity to the cages, so

**Table 1 Ethogram of behaviours recorded. Four rats were housed in each cage so each behaviour was recorded as the number of rats performing that behaviour at any one time, both before and after cage cleaning. If a skirmishing bout occurred during a formal observation period, the observer recorded the specific components of the behaviour. Because skirmishing was rare and of particular interest, it was also recorded whenever it was observed, giving a total number of bouts per cage. Similarly allogrooming was recorded whenever it occurred.**

Behaviour	Description
<i>Maintenance behaviours</i>	
Grooming	Licking, scratching or biting of own fur, or stroking forepaws over pelage.
Eating	Eating rat chow or seeds scattered in bedding.
Drinking	Drinking water.
Urinating	Urinating.
Defaecating	Defaecating.
<i>Inactive behaviours</i>	
Solitary resting	Lying down, moving very little or not at all, with eyes closed or half-closed.
Huddling	Resting as above, but in physical contact with one or more cage-mates.
<i>Locomotory behaviours</i>	
Walking	Walking around the cage.
Running	Running and jumping.
Rearing	Standing on the hind legs with the forepaws raised.
Climbing bars	Climbing along the cage bars.
<i>Other behaviours</i>	
Standing	Standing still.
Bar-biting	Biting the bars of the cage.
In shelter	With the head, at least, inside the Des-Res shelter. It was usually not possible to observe the rats' behaviours in the shelters.
Under hopper	With the head, at least, under the food hopper.
Digging	Manipulating the substrate with the fore-paws.
Mouthing bedding	Manipulating the substrate by nibbling or pushing with the snout.
<i>Social behaviours</i>	
Allogrooming	Grooming another rat using the forepaws or snout.
Social sniffing	Sniffing with the snout directed towards any area of another rat's body, and within approximately 2 cm of it.
Skirmishing	Chasing, boxing, wrestling, pinning-down, biting or mounting another rat (Meany & Stewart 1981; Barnett <i>et al</i> 1982; Pellis & Pellis 1987). Skirmishing bouts usually included several of these components.

the rats would have been subjected to the presence of humans at these times, even without the observer being present. All behavioural and subjective observations were carried out by the same observer (C Burn) to ensure consistency between results.

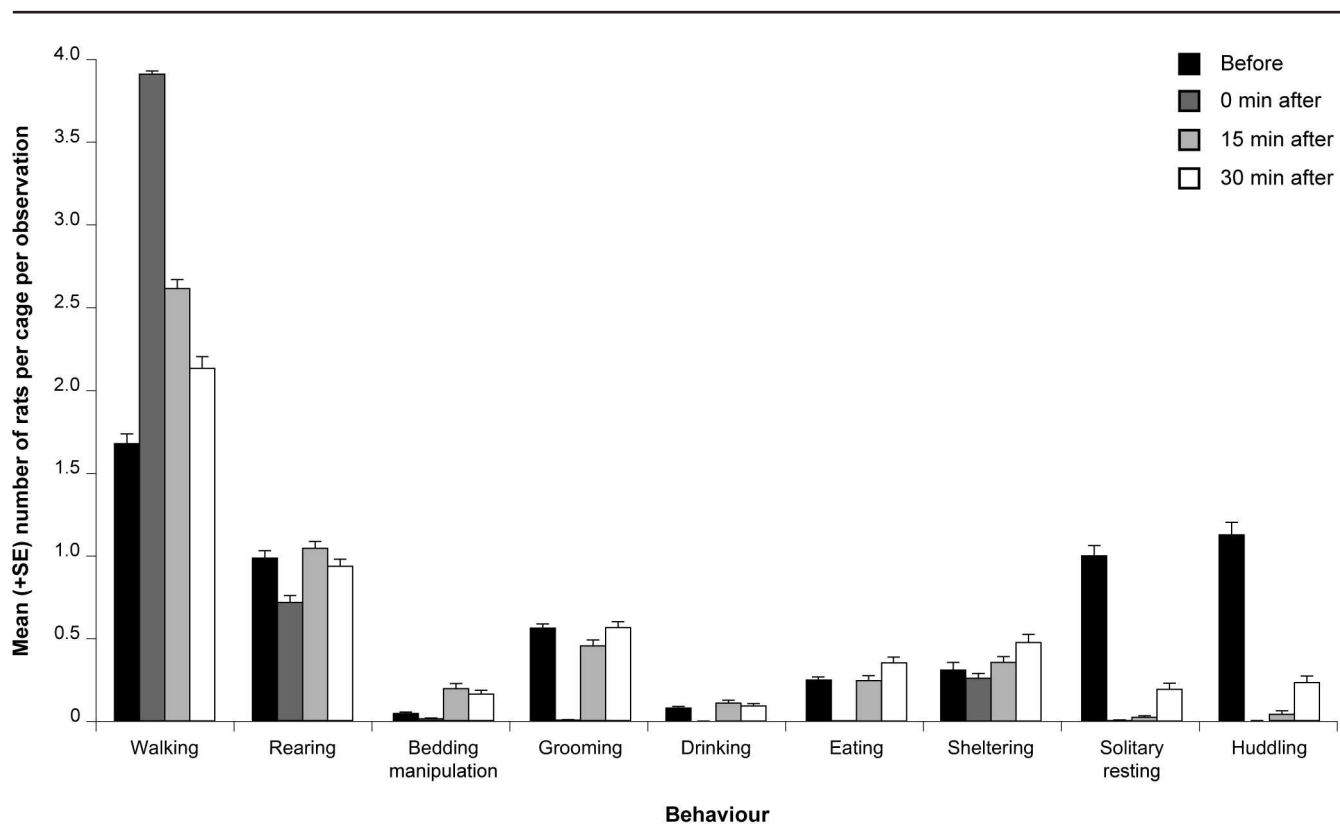
### Chromodacryorrhoea scoring

Because of the intensity of the behavioural observations, chromodacryorrhoea had to be scored after the observations were completed, which was in practice 35–45 min after each cage had been cleaned, and at the corresponding time on the day before cleaning. The observer was officially blind to the treatment groups. However, on the day before cleaning the degree of soiling in each cage made the treatment groups obvious in some cases. The rats were attracted to the front of the cage by gently tapping a pen against the bars and by the observer calling to them. The noses and eyes of the rats could then be easily subjectively scored as follows:

- 0 — No visible chromodacryorrhoea;
- 1 — One small (< 1 mm in diameter) speck of chromodacryorrhoea;
- 2 — One larger speck or a few small specks of chromodacryorrhoea;
- 3 — A few large specks or many small specks of chromodacryorrhoea;
- 4 — Approximately 25–50% of the nose covered or the eye surrounded by chromodacryorrhoea;
- 5 — More than 50% of nose covered in chromodacryorrhoea or eye surrounded by it.

The nose and each eye were scored separately, so each rat could attain a maximum score of 15, and each cage a maximum of 60. In addition, the area (in cm<sup>2</sup>) of chromodacryorrhoea that was visible as pale pink smudges on the fur was estimated by physical examination after observations had been completed on the day of cleaning.

Figure 1



The mean (+ standard error) number of rats per cage per observation that performed each behaviour before and after cage cleaning. After cleaning, behaviour is separated according to whether it was observed immediately after the cage had been placed back into the rack after cleaning or 15 or 30 min afterwards. Although the behaviours were also recorded at the corresponding intervals on the day before cleaning, a mean value is presented because the rats were not disturbed and the intervals did not differ from each other. Urination, defaecation, allogrooming, running, climbing bars, standing, bar-biting, and social sniffing were observed too rarely to be statistically analysed, so they are not included in the graph. 'Bedding manipulation' includes both 'digging' and 'mouthing bedding'; 'sheltering' includes 'in shelter' and 'under hopper' because these rare behaviours appeared highly correlated with each other.

### Statistical analyses

Data were summarised by a mean value per cage over the entire study period, unless age effects were of interest. The three observations taken on the day before each cleaning did not differ from each other so they were pooled into a single 'baseline' value for each cage. The observations taken after cleaning remained separate because the behaviours did change with time since cleaning.

Nine of the instantaneously observed behaviours were frequent enough to be analysed statistically. A principle components analysis was used to condense the behavioural data, but correlations between most behaviours were weak and did not load onto obviously meaningful axes (apart from an 'activity' axis with an Eigen value of 3.34). Therefore, each behaviour was analysed separately, apart from certain highly correlated behaviours that were summed in order to help reduce the number of tests performed: 'solitary resting' and 'huddling' were summed to give a measure of total 'resting'; and 'in shelter' and 'under hopper' were summed to give total 'sheltering'. In addition, 'digging' and 'mouthing bedding', which were too

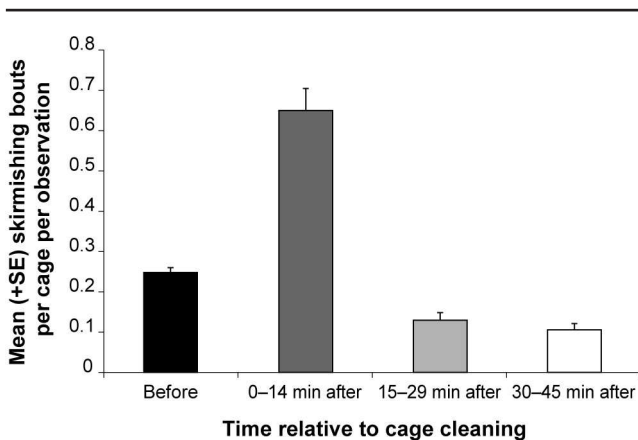
rare for statistical analysis alone, were often difficult to distinguish during observations and were summed to form 'bedding manipulation'.

Data were square-root transformed for parametric analysis where necessary. Paired *t*-tests (or Wilcoxon signed-ranks tests for the non-parametric data) were used to compare baseline behaviour frequencies with those (a) immediately after cage cleaning to assess the immediate impact of cleaning and (b) 30 min after cleaning to assess which behaviours were still affected 30 min after cleaning.

To test whether the frequency of cage cleaning affected the magnitude of peak post-cleaning skirmishing (skirmishing frequency immediately after cleaning / baseline skirmishing frequency), a general linear model (GLM) was used. Because aspen bedding was not used in all cages, because of the cleaning frequencies, only Alpha-Dri treatments were tested. The model included cage cleaning frequency, rat stock, and animal unit, plus their interactions as fixed factors. The same model was used to assess how cage cleaning frequency affected the proportion of rats that had started resting 30 min after cleaning, compared with the

**Table 2** Effects of cage cleaning on instantaneously observed behaviours immediately after cleaning and 30 min afterwards (ns = not significant).

Behaviour	Direction of effect	T value (n = 80)	P value
<i>Immediate effect of cage cleaning</i>			
Grooming	Decreased	22.05	< 0.001
Eating	Decreased	16.38	< 0.001
Drinking	Decreased	8.89	< 0.001
Resting	Decreased	16.27	< 0.001
Walking	Increased	38.58	< 0.001
Rearing	Decreased	4.46	< 0.001
Sheltering	None	1.14	ns
Manipulating bedding	Decreased	3.60	0.001
<i>Effect 30 min after cleaning</i>			
Grooming	None	0.07	ns
Eating	Increased	3.67	< 0.001
Drinking	None	0.90	ns
Resting	Decreased	14.70	< 0.001
Walking	Increased	5.87	< 0.001
Rearing	None	0.95	ns
Sheltering	Increased	3.31	< 0.001
Manipulating bedding	Increased	4.61	< 0.001

**Figure 2**

Skirmishing frequency before and after cage cleaning. The mean (+ SE) number of skirmishing bouts observed per cage per observation before cage cleaning and at intervals after it. Skirmishing was observed significantly more frequently during the 15 min following cleaning than on the day before cleaning ( $F_{2,158} = 112.29$ ,  $P < 0.001$ ), but thereafter it decreased to below baseline levels ( $t = 2.54$ ,  $df = 79$ ,  $P = 0.013$ ). All the skirmishing observed was non-aggressive and play-like.

baseline, and any relative change in chromodacryorrhoea scores before and after cleaning. To examine the effects of bedding-type on the frequency of bedding manipulation, the fortnightly (Alpha-Dri only) treatment was excluded from analyses, and a Mann-Whitney  $U$  test was used because the

data did not fit the assumptions of parametric tests. The effect of age on skirmishing levels was examined using repeated measures GLMs, including 'cage' and 'time', as well as the above factors. Correlations between chromodacryorrhoea, resting, grooming and skirmishing, and between skirmishing and squeaking were tested using regression analyses. The software was Minitab™ version 13.20 (Minitab Ltd, Pennsylvania, USA).

Because of the large number of statistical tests necessary here, results should be treated as exploratory, therefore requiring confirmation using more specific studies (Bender & Lange 2001).

## Results

### General effects of cage cleaning on behaviour

General effects of cage cleaning on the instantaneously observed behaviour are shown in Figure 1, and the statistical results are shown in Table 2; the effects of cage cleaning on skirmishing are shown in Figure 2.

### Behaviour immediately after cage cleaning

Immediately after cage cleaning, walking significantly increased above baseline levels ( $t = 38.58$ ,  $n = 80$ ,  $P < 0.001$ ), whereas almost all other behaviours significantly decreased; only sheltering remained unchanged. Skirmishing during the first 15 min after cleaning was much higher than on the day before cleaning ( $F_{2,158} = 112.29$ ,  $P < 0.001$ ) (Figure 2). All the skirmishing observed was more similar to play-fighting than to serious aggression

(Pellis & Pellis 1987). Specifically, the nape of the neck was the target of attack rather than the rump, biting was only observed once and it caused no injury, and pilo-erection was never observed. There were positive correlations between squeaking and skirmishing on the day before cleaning ( $F_{1,78} = 98.68$ ,  $r^2 = 0.56$ ,  $P < 0.001$ ) and during the peak in skirmishing just after cleaning ( $F_{1,77} = 36.01$ ,  $r^2 = 0.32$ ,  $P < 0.001$ ) (Figure 3).

### Behaviour 30 min after cage cleaning

After 30 min, the number of rats walking still remained higher than the baseline levels, as did the number of rats eating, manipulating bedding and sheltering. To assess whether the increases in bedding manipulation and feeding were simply a product of the rats being generally more active after cleaning than before, a paired  $t$ -test was used to compare the proportions of active rats that were manipulating bedding or feeding before cleaning and 30 min after cleaning; a significantly higher proportion of rats manipulated bedding after cleaning than before it ( $t = 4.61$ ,  $n = 80$ ,  $P < 0.001$ ) but there was no such difference in the proportions of rats feeding. To check whether sheltering and eating were confounded due to rats both sheltering and eating under the food-hopper, a Wilcoxon test was carried out with only the (non-parametric) 'in shelter' data; this remained significantly higher than the baseline (medians: 0.25 rats per cage after cleaning and 0.03 before,  $n = 80$ ,  $P < 0.001$ ).

Resting was the only instantaneously observed behaviour that remained at a lower frequency after cleaning than on the day before; however, the level of skirmishing also decreased to below that observed on the day before cleaning ( $t = 3.46$ ,  $n = 80$ ,  $P = 0.001$ ).

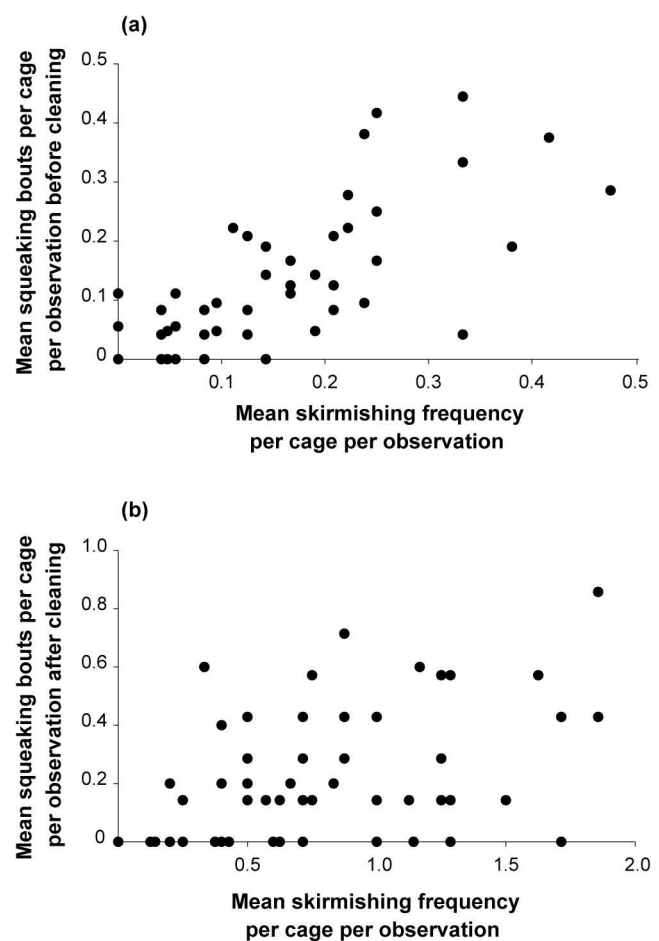
### Effects of cleaning frequency on post-cleaning skirmishing and resting

There was a positive relationship between the frequency of cage cleaning and the proportion of rats resting 30 min after cage cleaning compared with their baseline levels ( $F_{2,24} = 11.96$ ,  $P < 0.001$ ), that is, rats returned toward their baseline resting levels most quickly in the cages that were cleaned twice per week, and least quickly in the cages that were cleaned once per fortnight (Figure 4). The frequency of cleaning had no effect on the magnitude of the post-cleaning peak in skirmishing.

### Chromodacryorrhoea

Chromodacryorrhoea was observed on the nose at some point in every cage, whereas it was only observed around the eyes in 30 of the 80 cages. The amount of chromodacryorrhoea on the fur did not correlate with nose or eye scores, and did not differ significantly with any of the variables tested. Because nasal chromodacryorrhoea is also known to increase even in response to mild stressors (Mason *et al* 2004), nasal chromodacryorrhoea was the main response variable used in this study. General findings relating to chromodacryorrhoea are described in detail elsewhere (C Burn DPhil Thesis Unpublished).

Figure 3



Mean skirmishing and squeaking were positively correlated (a) on the day before cage cleaning ( $F_{1,78} = 98.68$ ,  $r^2 = 0.56$ ,  $P < 0.001$ ), and (b) during the skirmishing peak immediately after cleaning ( $F_{1,77} = 36.01$ ,  $r^2 = 0.32$ ,  $P < 0.001$ ).

Chromodacryorrhoea was higher on the day before cage cleaning than after cleaning ( $t = 3.69$ ,  $n = 64$ ,  $P < 0.001$ ) (Figure 5). Cleaning frequency had no significant effect on chromodacryorrhoea on either day, and did not affect the magnitude of the change in chromodacryorrhoea after cleaning compared with the day before cleaning. Chromodacryorrhoea did not correlate with resting levels, skirmishing or grooming, which might have reduced the amount of visible chromodacryorrhoea.

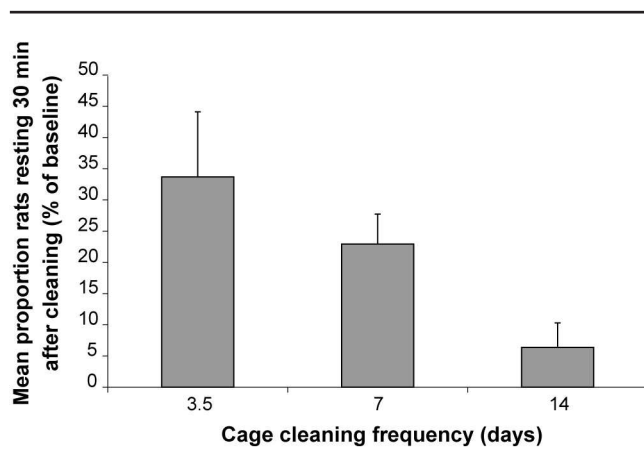
### Bedding manipulation

The rats manipulated aspen woodchips more than Alpha-Dri (medians: 0.10 and 0.17 rats per cage respectively;  $df = 31$ ,  $P = 0.032$ ), which appears to have been mainly attributable to mouthing rather than digging (Figure 6).

### Discussion

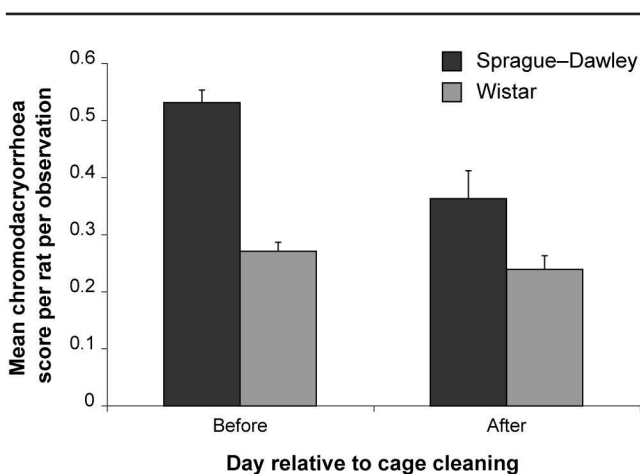
This study found that cage cleaning increased the general activity of rats above baseline levels for the full 30 min observation period and that the number of rats resting had

Figure 4



The effect of cage cleaning frequency on the mean (+ SE) proportion of rats resting 30 min after cleaning relative to their baseline resting frequencies on the day before cleaning. A higher proportion of rats rested within 30 min of cage cleaning if their cages were cleaned more frequently ( $F_{2,24} = 11.96, P < 0.001$ ).

Figure 5



The mean (+ SE) chromodacryorrhoea scores of rats on the day before cage cleaning compared with immediately after cleaning. Chromodacryorrhoea was significantly lower 45 min after cage cleaning than on the day before it ( $t = 3.69, n = 64, P < 0.001$ ). Rat stock is also included because it had a significant effect on chromodacryorrhoea (C Burn DPhil Thesis, Unpublished).

not returned to pre-cleaning levels at the end of this 30 min observation period. This is in agreement with other studies on the acute effects of cage cleaning (Saibaba *et al* 1996; Schnecko *et al* 1998; Doerning 1999; Duke *et al* 2001; Sharp *et al* 2002a; Sharp *et al* 2002b), some of which have shown this increase to persist for at least 2 h after cage cleaning (Saibaba *et al* 1996; Schnecko *et al* 1998; Sharp *et al* 2002b). Our observations show that the increased general activity included walking, bedding manipulation and feeding (the increase in sheltering could have been active or inactive). It is possible that some of the rarer behaviours (eg defaecation [Saibaba *et al* 1996] and perhaps

social sniffing and allogrooming, which appeared to be associated with skirmishing), were also affected by cleaning but we observed no obvious patterns. Because these behaviours were rare, a more intensive observational schedule would be required for any conclusions to be drawn about them.

After cage cleaning there was a marked increase in skirmishing to above pre-cleaning levels, but the effect was transient and after 15 min skirmishing returned to below pre-cleaning levels, as previously found in mice (Gray & Hurst 1995; Van Loo *et al* 2000). However, unlike in mice, all the skirmishing observed was non-aggressive and appeared similar to the non-injurious skirmishing between familiar rats described by Pellis & Pellis (1987, 1992) and Pellis *et al* (1993). Skirmishing did correlate positively with audible squeaking, but we know of no scientific evaluation of the significance of audible squeaking as a welfare measure, or its functional significance in rats. In addition, over the course of the experiment, wounds were always superficial and did not show any relationship with skirmishing frequency (Burn *et al* 2006 in press). In fact, most wounds (42 of 54) were found on the tail and therefore could have been self-inflicted. This contrasts with the superficially similar post-cleaning peak in skirmishing that occurs between mice during which injuries are a common problem, particularly in aggressive strains (Van Loo *et al* 2003). Cleaning frequency did not affect the magnitude of the post-cleaning peak in skirmishing, suggesting that the peak is neither caused by any relative change in the olfactory environment nor influenced by how habituated rats are to disturbance.

In contrast, more frequent cleaning was associated with a higher proportion of rats that had returned to resting behaviour 30 min after cleaning. This finding is in agreement with Duke *et al* (2001), who found that rats cleaned once per fortnight showed a small but significant increase in general activity after cleaning compared with those cleaned once per week. This may be because rats cleaned more frequently habituate to the disturbance and settle down more quickly after cleaning than rats that are cleaned less frequently, or because the change in the within-cage-environment effected by cage cleaning is greater for the animals that are cleaned once per fortnight (which have much dirtier cages prior to cleaning), stimulating more activity. Again, however, the welfare implications are not clear: frequent mild disturbance could be either better or worse than less frequent but more disruptive disturbance. In addition, the welfare impact would depend on whether that disturbance is aversive to the rats, or if it is neutrally or even positively stimulating.

The finding that chromodacryorrhoea was higher the day before cage cleaning than after cleaning could suggest that having a soiled cage was more stressful or aversive than the cleaning procedure itself. If that were the case, the lack of any effect of cleaning frequency on chromodacryorrhoea suggests that just a few days of soiling caused the rats as much distress as two-weeks of soiling. This could be the case if rats are adapted to using specific latrines in the wild, as mice do (Sherwin 1996); wild rats would then be able to



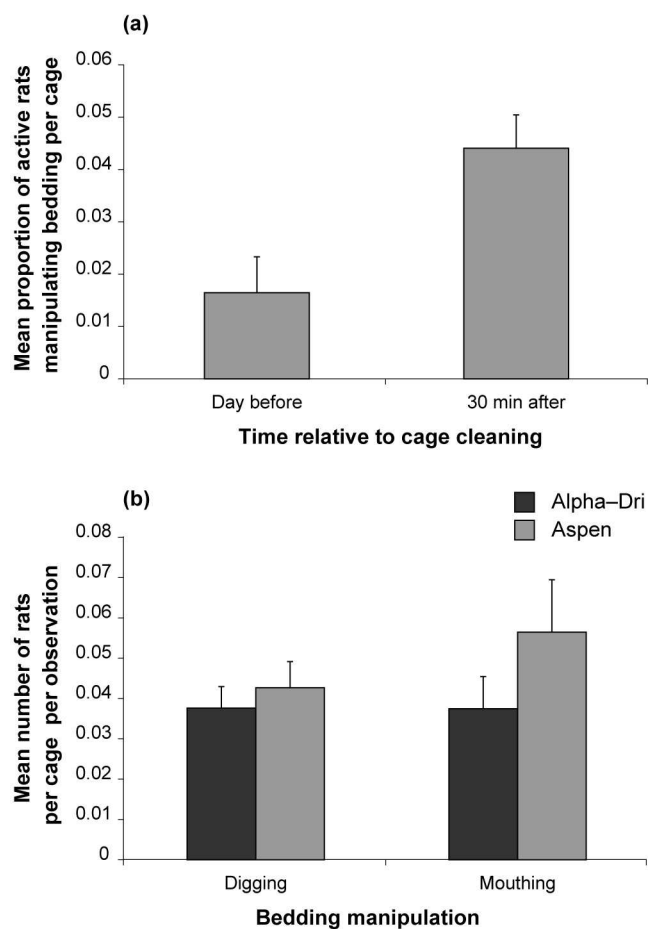
choose whether or not to be in the proximity of their urine and faeces. However, we know of no empirical evidence that wild rats use latrines, although some pet rats establish toilet areas in large cages (Fox 1997; Bulla 1999). Furthermore, pilot studies in our laboratory found that 9 pairs of adult Lister-hooded rats showed no avoidance or preference for their own soiled substrate over clean bedding, even after three weeks (C Burn DPhil Thesis Unpublished).

Another explanation for the decrease in chromodacryorrhoea post-cleaning could be that the rats groomed the secretion away from their eyes and nose on to their fur after cage cleaning but before it could be scored by the observer. Although grooming did not appear to increase above baseline levels after cleaning in our study, more frequent but curtailed grooming bouts do occur in novel or stressful situations (perhaps including cage cleaning) compared with situations where rats are less vigilant (Komorowska & Pisula 2003). Although our instantaneous observations did not allow us to quantify or verify this effect, an increased grooming frequency after cleaning was observed by Duke *et al* (2001). In further support of this hypothesis, subsequent studies within our research group have found that handling-induced chromodacryorrhoea peaks approximately 10 min after handling, and that it begins to decrease after 15 min when rats start to groom again (C Burn DPhil Thesis Unpublished). Because handling is a necessary component of cage cleaning, it is possible that recording chromodacryorrhoea 35–45 min after cage cleaning, as in this current study, was too late to observe the peak chromodacryorrhoea response. Further work would therefore be necessary to discover whether or not chromodacryorrhoea increases after cage cleaning, aside from handling *per se*, and whether the extent of any increase is influenced by how frequently rats' cages are cleaned. For example, cage cleaning could be simulated but the rats returned to their original cage. However, it is worth noting here that as well as finding no evidence that rats prefer soiled cages, our own work has also shown that frequently cleaned rats do not differ in growth rates or general health measures from less frequently cleaned rats (Burn *et al* 2006 in press; C Burn DPhil Thesis Unpublished).

The rats manipulated the bedding more after cage cleaning than before cleaning and this was not due to simply being generally more active. This manipulation included both an increase in digging and mouthing the bedding. This is surprising because rats are more active on home-cage bedding than fresh bedding (Buelke-Sam *et al* 1984), and their own soiled bedding has an anxiolytic effect in a light-dark preference test (Richardson & Campbell 1988). However, a previous study showed that digging activity increased with the novelty of the arena containing the substrate (Schultz 1972), and the author suggested that digging might indicate the rats' aversion to the novel area, perhaps being an escape-motivated behaviour. Alternatively, the rats may dig and mouth bedding more after cleaning because they prefer to manipulate bedding when it is clean rather than when it is soiled.

Bedding manipulation was also observed more frequently on aspen bedding than on Alpha-Dri, mainly because of an

Figure 6



Bedding manipulation relative to cage cleaning and bedding type. (a) The mean (+ SE) proportion of active rats manipulating bedding per cage per observation the day before and 30 min after cage cleaning. A significantly higher proportion of active rats manipulated bedding after cleaning than before it ( $t = 4.61$ ,  $n = 80$ ,  $P < 0.001$ ). (b) The mean (+ SE) number of rats manipulating aspen and Alpha-Dri bedding per cage per observation. Bedding manipulation is separated according to whether it involved digging or mouthing the bedding, both of which were observed too rarely to be statistically analysed separately. Rats manipulated aspen chip bedding more than Alpha-Dri ( $df = 31$ ,  $P = 0.032$ ) and the graph suggests that this was mostly an effect of mouthing the bedding rather than digging in it.

increase in mouthing. This increased mouthing of the aspen bedding is likely to have included actual ingestion of that bedding because rats kept on aspen had correspondingly higher body weights than those on Alpha-Dri (Burn *et al* 2006 in press); however, we cannot confirm this because the amount of bedding eaten or its calorific value was not quantified. In addition to any gustatory or nutritional aspects of the two beddings, their particulate qualities might also have influenced general bedding manipulation because wild rats dig more burrows in loose substrates than in more compact substrates (Lore & Flannelly 1978). Therefore, the rats may have found it easier to manipulate the aspen bedding because the woodchip particles appeared to be more loosely

packed than in Alpha-Dri and did not appear to become as compacted. We know of no studies that have compared the relative preferences of rats for Alpha-Dri or anything similar to it, but some preference tests have shown that mice and rats prefer aspen over a variety of other beddings (Mulder 1975; Odyne *et al* 1991; Ras *et al* 2002). However, rats housed on Alpha-Dri bedding sneeze less and have less severe background lung pathology (interstitial pneumonia) than those kept on aspen (Burn *et al* 2006 in press); therefore, for long-term housing in conventional units, where respiratory infections are a risk, the use of aspen bedding would not be advisable even if it is preferred. The rats were more often seen inside sheltered areas of the cage after cleaning than before, despite being more active. This was the case even when sheltering under the food hopper was excluded from the analyses, showing that it was not simply an effect of the increased frequency of feeding after cage cleaning. One explanation could be that the rats, having been awakened by the cleaning process, chose to move to the shelters to avoid the light, whereas undisturbed rats (on the day before cleaning) remained in the exposed positions in which they had been resting since the dark period. Alternatively, the disturbance from cage cleaning might have motivated the rats to seek shelter from human interference or from the novel environment of their new cage. In either case, this finding reinforces the evidence that the provision of shelter enables rats to choose their environment appropriately, allowing them to take refuge from light or other aversive stimuli (Townsend 1997; Manser *et al* 1998; Patterson Kane 2003; De Villiers *et al* 2004).

#### Animal welfare implications

We have described in detail the behaviours that are influenced by cage cleaning in rats, and have confirmed that the peak of skirmishing, which appears to resemble aggression in mice, is non-aggressive and play-like in Sprague-Dawley and Wistar rats. We have found no behavioural evidence to suggest that the increased post-cleaning activity was an acute stress response that corresponded to a negative effect of cage cleaning on rat welfare — a finding consistent with our other work on these subjects, which has shown no long-term effect of increased cage cleaning frequency on growth rates or general health, and no preference for soiled over clean cages (Burn *et al* 2006 in press; C Burn DPhil Thesis Unpublished). Chromodacryorrhoea could potentially be useful in further determining whether cage cleaning is a stressful experience in rats or not, but it should be observed within approximately 15 min of cage cleaning, before the secretion is groomed away. Clarification of this is important to ensure that the welfare of rats is not chronically decreased through the constant presence of their own urine and faeces without the opportunity for the use of latrine areas. The frequency of cage cleaning did not affect the magnitude of the skirmishing peak or the decrease in chromodacryorrhoea, but more frequent cleaning led to rats settling down more quickly after cleaning than if they were less habituated to the process. It would be interesting to know whether cage cleaning frequency influences the stress response of rats to other, more novel situations; for example, whether the more

frequently cleaned rats are habituated to novelty and change generally, or just specifically to cage cleaning events. Overall, however, there appears to be no clear welfare benefit to using one cage cleaning frequency over another.

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