

The assessment of bar chewing as an escape behaviour in laboratory mice

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Abstract

The ability to measure objectively how an animal perceives its home environment is essential for improving the housing and husbandry conditions of laboratory animals. Chewing at cage bars by a rodent may reflect the animal's desire to escape from its home cage and thus provide a measure of the relative aversiveness or inadequacy of different housing conditions from the animal's viewpoint. To assess whether bar chewing by laboratory mice is an escape behaviour, adult male and female ICR-(CD-1) mice were housed individually or in same-sex groups of three in modified shoebox-type cages. Cages had two sets of external bars in the side walls, an equivalent set of bars fixed internally and a Perspex lid. One set of external bars opened daily, allowing the mice to escape into a larger arena. All mice showed a strong preference for chewing at external bars over those that were internal to each cage. After one week of experience, mice also preferred the external bars that opened daily to those that did not open. Behaviour directed towards the cage lid declined over time as the mice experienced the new escape route in the cage side. Interest in the external bars correlated positively with time since last escape. Results confirm that bar chewing reflects an attempt to escape the cage and explore the surrounding area and may provide a suitable behavioural measure of perception of the cage environment for use in welfare assessment.

Keywords: animal welfare, bar chewing, escape, mice, welfare assessment

Introduction

An objective measure of animal welfare is an essential prerequisite to the design of housing and husbandry conditions that will maximise the welfare of laboratory rodents. Understanding an animal's response to its home environment is particularly important because this is where it will spend most of its life. However, assessing the needs and motivation of a captive animal from the animal's point of view whilst avoiding anthropomorphism is highly problematic. Although methods for assessing welfare exist, none are without their problems (Dawkins 1990). An ideal method should combine accuracy with ease of use, be non-intrusive, unambiguous and allow welfare to be measured within the animals' home cage without disturbance.

One very useful approach for highlighting specific requirements within the home cage is preference testing (Blom *et al* 1996; Sherwin 1996; Van de Weerd *et al* 1998a,b). However, the use of preference tests alone has many limitations. In particular, preference tests reveal little about the animal's perception of its cage as a whole, and a preference between two options does not mean that the animal has an aversion to the non-preferred option (Rushen 1993). Preference testing may also lead to animals continually choosing environments that are bigger and better, yet are impractical for use in a laboratory. Another problem is that preference tests show only what the animal wants at a particular moment in time (Dawkins 1990) and animals may

make a choice irrespective of whether this decision jeopardises their long-term survival or welfare (Dawkins 1988). If a choice is made based on the information available at a particular moment in time, this does not necessarily mean that the animal would want to spend all of its time in the chosen environment. Alternatively the animal may simply be unaware that it is making a choice. Further, a preference for a particular resource does not necessarily mean that an animal's welfare will be compromised if it does not have that resource. In natural situations, animals may be unlikely to obtain their ideal choice of food or nest site. However, if an animal finds itself in a position that may be harmful or undesirable (eg lacking in an important resource) and its welfare is compromised by this, then it will make attempts to move away from that situation to find the required resource or better conditions elsewhere. This can be termed 'escape behaviour', as actual escape from the cage is necessary to find the resource or to discover a better environment. Measurement of escape behaviour might provide a more feasible and accurate assessment of welfare than measurement of preferences.

Among captive rodents, bar chewing may be one such escape behaviour. In natural situations, mice and rats gnaw at physical structures in order to gain access into a suitable environment, particularly when attempting to enter buildings, or to reach a goal such as food or a safe hiding place. Such gnawing or chewing behaviour is also seen frequently

in captivity, where it is predominately directed at the cage bars and can be carried out for up to 90% of the active period of laboratory mice (Lewis 2003). However, laboratory cages have been designed with stainless steel bars specifically to prevent animals chewing through them and escaping. From the human perspective, therefore, bar chewing is in effect functionless and has been labelled as a functionless stereotypic behaviour (Wurbel *et al* 1996) or a coping response (Cooper & Nicol 1996). However, from the animal's perspective, the amount of time spent chewing at cage bars may represent the level of the animal's desire to leave its home cage (Hurst *et al* 1999; Nevison *et al* 1999a).

There is already some evidence in support of the hypothesis that bar chewing by laboratory rodents relates to an attempt to escape. Wurbel and Stauffacher (1997) found higher levels of exploratory and bar-related behaviour among precociously weaned mice, perhaps reflecting an attempt to escape the cage in order to return to the mother to suckle. This translated into increased levels of bar chewing among older animals, although Wurbel *et al* (1996) defined chewing as stereotypic behaviour. Enriching the environment can reduce the levels of bar-related behaviours (Wurbel *et al* 1998; Nevison *et al* 1999b). Differences in the cage environment also affect the level of bar chewing among rats. Cages that restrict social interaction with neighbouring animals can lead to a higher incidence of bar-chewing behaviour (Hurst *et al* 1997, 1998). Hurst *et al* (1996, 1999) also found that the level of bar chewing and other escape-related behaviours shown by individual rats correlates strongly with social conflict experienced within caged groups and pathophysiological indicators of stress, including tissue pathology and elevated corticosterone and immunoglobulin G (IgG) levels.

However, none of these studies provides direct evidence that bar chewing is escape-related rather than just a symptom of frustration or indeed a coping response to a stressful situation. It is also possible that bar-chewing behaviour may begin after weaning as a frustrated escape attempt but become stereotypic in older mice as they learn that escape is not possible. Nevison *et al* (1999a) were the first to test explicitly the hypothesis that bar chewing is an escape response in laboratory mice. They showed that male ICR-(CD-1) mice housed in groups prefer to chew bars that allow olfactory contact with the external environment and that open during routine husbandry. However, bar location was a confounding factor in their study. Mice were presented with a choice between two sets of bars, one in the side wall of the cage and one in the roof, but only showed a preference for chewing bars that opened when these were located in the side wall of the cage. This may have been because of different energetic costs of interacting with the roof and with the side bars. The authors also only considered the behaviour of male mice that had been housed in experimental cages from birth, and no studies appear to have examined bar chewing among animals housed in social isolation.

In order to assess whether levels of bar chewing can be used as a simple and practical measure of welfare in a standard laboratory situation, it still remains to be shown whether bar chewing really does reflect an animal's immediate desire to

leave its cage, whether this depends on previous experience, and whether there are differences in this behaviour according to the sex of the animals or housing in social isolation or in groups. The aim of this study was to determine whether laboratory mice of both sexes chew the bars in order to escape from their cage and how this is influenced by experience. Male and female ICR-(CD-1) mice were housed as adults in cages that contained an internal set of bars and two external sets of bars in the cage sides, one of which was opened daily to allow escape. It was predicted that if mice chew at cage bars as an attempt to escape, they will direct much more chewing behaviour towards external bars, and particularly those through which they have experience of escaping, than towards bars internal to the cage. Alternatively, if bar chewing represents a functionless stereotypy, a coping response or simply a behavioural need to gnaw, then mice should chew any barred surface in the cage.

Methods

Subjects and maintenance

Two weeks prior to the start of the experiment, 24 male and 24 female ICR-(CD-1) mice (Harlan, Bicester, UK) aged four to five weeks were established in single-sex groups of three or were housed individually, to give six replicates of each sex and group or individual housing condition. The animals were housed in wire-lidded standard M3 cages with polypropylene bases (48 cm × 15 cm × 13 cm; North Kent Plastic Cages Ltd, Kent, UK) containing sawdust bedding and shredded paper nesting material. Cages were cleaned twice per week. Throughout the study, all mice were provided with food (TRM 9607 Rat and Mouse Diet, Harlan-TekladTM, Bicester, UK) and water *ad libitum*. Cages were interspersed evenly on the rack to avoid positional sex bias. The mice were maintained on a 0900h–2100h reverse light cycle and all observations were made in the dark period under dim red lights.

Experimental housing conditions

At the age of six to seven weeks, both group-housed and singly housed mice were transferred to experimental cages. Standard M1 polypropylene mouse cages (33 cm × 15 cm × 13 cm; North Kent Plastic Cages Ltd, Kent, UK) were modified by cutting out two wall sections (12 cm × 9 cm) in the long side walls to which two sets of bars were fitted (external bars). Another set of bars was fitted inside the cage (internal bars; see Figure 1). The wire lid was replaced with a sheet of clear Perspex, the water bottle was attached to the outside wall of the cage with the nozzle protruding in through the front wall, and food was placed inside the cage daily. One set of external bars could be slid open to allow the mice to escape from the cage at regular intervals (half of the cages opened on the right-hand side and half on the left). Cages were transferred individually into the centre of a large arena (60 cm × 60 cm × 55 cm) where the bars were opened for 5 min each day allowing the mice to escape and explore the arena. After 5 min the mice were placed back into the cage and the cage returned to the rack. The arena was cleaned with ethanol following each

escape attempt. Cardboard barriers were placed between neighbouring cages so that visual contact was not possible.

Behavioural observations

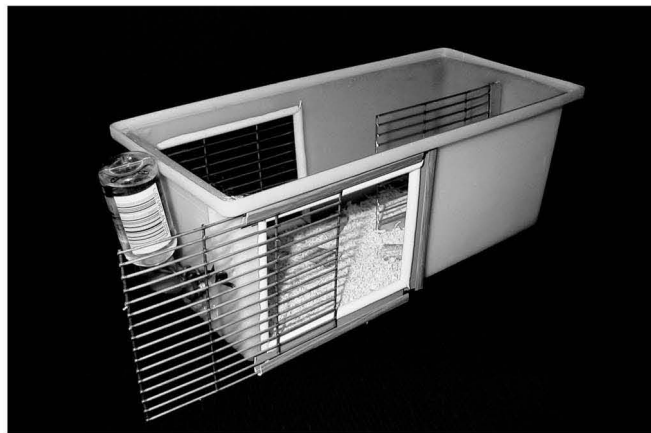
Each animal was marked in one of three distinctive patterns with fur dye (Clairol nice'n'easy™ Shade 122: Bristol-Myers Co Ltd, Uxbridge, UK). Behaviour was assessed on day one, when the mice were first introduced into experimental cages, and on day eight after a week in their cages. To observe the initial reaction to the three sets of bars, animals were videoed for 1 h immediately after being placed into the experimental cage at 1000h (session 1). At the end of this period, animals were allowed to escape through the designated external bars for 5 min. To gauge the initial reaction to escape, mice were then placed back into the cage through the opening bars, the bars were shut and behaviour recorded for another hour (session 2). Animals were videoed again 3 h after they had experienced escape in order to gauge a more long-term response to the escape (session 3). Animals were allowed to escape from their cage for 5 min each day over the following six days, with all husbandry procedures carried out through the designated opening side, before repeating the same set of three observation periods on day eight (sessions 4–6).

Thirty minutes of each observation session were used for behavioural analysis, starting when all of the mice in the observed group were active (ie not sleeping). In practice, this was always the first 30 min of each session. Both the location of each mouse and its behaviour were transcribed from videotapes, using instantaneous samples taken at 15 s intervals to give 120 observations per mouse per session. Location was recorded as one of four possibilities: next to the opening external bars (nose within 1 cm of the bars); next to the non-opening external bars; next to the internal bars; or anywhere else within the cage. The behaviours recorded were bar chewing (gnawing or mouthing the cage bars); bar-related behaviour (any other behaviour directed at the cage bars including pulling, shaking, sniffing and climbing); roof-related behaviour (any interaction with the cage roof, such as pawing and sniffing); inactive (sleeping or sitting without movement); and other (any behaviour not listed above).

Data analysis

Data per session were averaged for mice within each caged group. Non-parametric analyses were used as most variables were not normally distributed (Kolmogorov-Smirnov tests, $P < 0.05$, conducted in SPSS Version 9.0). Specific Wilcoxon matched-sets tests (Meddis 1984) tested the hypotheses that mice would spend more time near to the external bars (either opening or non-opening) than near to the internal bars during each session, and that they would chew more at external than at internal bars. Separate tests assessed behaviour prior to experience of escape through the side bars (session 1) and behaviour after at least one experience of escape (sessions 2–6 combined). Specific Wilcoxon matched-sets tests also assessed whether mice spent more time chewing the opening than the non-opening

Figure 1



Modified M1 mouse cage fitted with a set of internal bars and two sets of external bars, one of which could be opened manually.

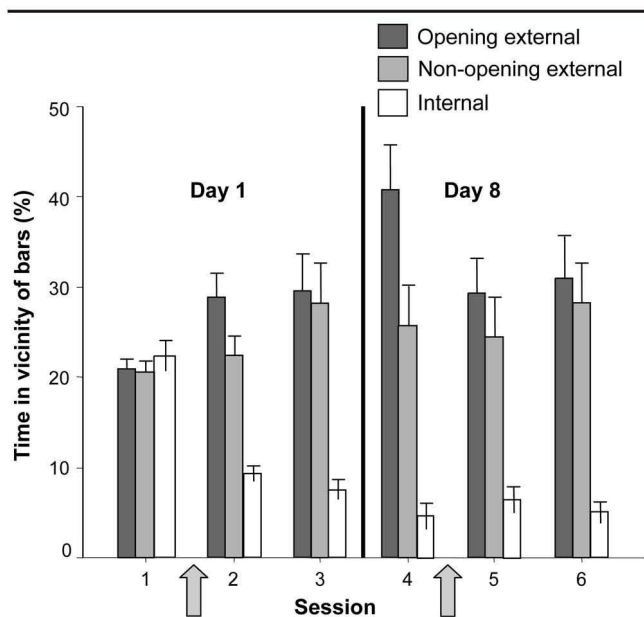
external bars. Non-parametric two-way ANOVAs (Meddis 1984) examined whether sex and group size had any effects either on the bias in bar chewing (the arithmetic difference in time spent chewing at the opening minus the non-opening external bars, or the difference in mean time chewing at the two sets of external bars minus time chewing at the internal bars) or on the total time spent near the cage bars. Changes in roof-related behaviour over time, and the effect of time since last escape on bar chewing, were assessed using the approximate test for trends and contrasts (Meddis 1984).

Results

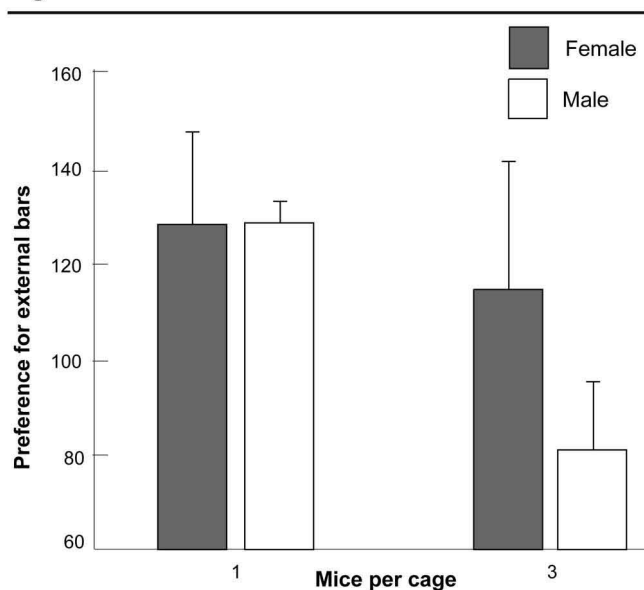
Preference for external cage bars

When mice first entered the experimental cage, there was no difference in total time spent near the internal bars compared to each of the external bars (session 1, $Z = 0.26$, not significant [ns], Figure 2) and there were no differences in this preference according to sex or group size (session 1, group size, $H = 1.20$, ns; sex, $H = 0.44$, ns; interaction between sex and group size, $H = 0.01$, ns). However, after escape experience, mice spent much more time in the vicinity of each of the external bars compared to the internal bars (sessions 2–6, $Z = 10.32$, $P < 0.001$; Figure 2). There was a significant interaction between sex and group size in this preference, since group-housed males showed a weaker preference for the external bars than did singly housed males or females housed singly or in groups (sessions 2–6, group size, $H = 0.33$, ns; sex, $H = 0.56$, ns; interaction between sex and group size, $H = 4.09$, $P < 0.05$; Figure 3).

Although mice showed no bias in the total time spent in the vicinity of internal or external bars before experience of escape through the external bars, a significant preference for chewing at external bars was evident even during session 1 ($Z = 4.21$, $P < 0.001$; Figure 4). This preference grew stronger once the animals had experienced escape, and chewing at the internal bars almost disappeared after the first experience of escape through external sidebars (sessions 2–6, $Z = 13.48$, $P < 0.001$; Figure 4).

Figure 2

Percentage of time spent in the vicinity of the three sets of bars in each session (mean \pm standard error per cage for all mice). Arrows indicate time of escape.

Figure 3

Bias in preference for the external bars (time near external bars minus time near internal bars) according to sex and housing density, for sessions 2–6 combined (mean \pm standard error per cage).

Escape route

In addition to choosing between internal and external bars, mice also had a choice between external bars that opened regularly and external bars that remained closed throughout the experiment. When mice first entered the experimental cage (session 1) they showed no preference for spending more time near ($Z = -0.34$, ns) or chewing ($Z = -0.44$, ns; Figure 2) the opening external bars. However, once they had experienced escape, mice spent more time chewing the opening bars (sessions 2–6, $Z = 2.67$, $P < 0.01$; Figure 4).

All mice showed a similar bias regardless of sex or group size (sessions 2–6, group size, $H = 0.33$, ns; sex, $H = 0.56$, ns; interaction between sex and group size, $H = 0.57$, ns). The bias towards the opening bars specifically concerned bar-chewing behaviour. There were no significant differences in total time spent near to the opening versus the non-opening external bars.

When the mice were first introduced into their experimental cage, they repeatedly stretched up to the cage lid, which had been the only previous route through which mice entered or left their cages during routine maintenance. Interaction with the roof declined significantly over time ($Z = 7.16$, $P < 0.001$; Figure 5), particularly after the first experience of escape through the sidebars of experimental cages. The levels of roof-related behaviour followed a similar pattern among singly housed and group-housed males and females.

Time since last escape

By day 8, mice had acquired repeated experience of escape through one set of external bars; comparison of the three sessions recorded on day 8 showed that mice spent an increasing proportion of time chewing the external bars according to the time since their last escape ($Z = 3.99$, $P < 0.001$; Figure 6). Again, data followed a similar pattern for all sex and group size classes.

Discussion

The results of this experiment provide strong evidence in support of the hypothesis that bar chewing is an escape behaviour in adult laboratory mice. On first entering the cage, the animals showed an immediate preference for chewing at bars in the cage sides that led to the external environment. This result is consistent with Nevison *et al* (1999a), who found that male mice of all ages preferred to chew external bars that were not backed by Perspex, which reduced cues from the external environment. In both cases, this response may have signified that mice were attempting to escape from the cage, but mice could also have been interacting with the cage bars in order to gain information from the external environment. However, once mice had experienced escape through one of two identical sets of external bars in this study, their preference for the external bars over the internal bars increased and a clear bias emerged between the two sets of external bars such that the mice spent more time chewing the external bars that opened daily. This bias towards opening bars could not be explained as an interaction with a view to gaining information, since both sets of bars were otherwise identical once closed.

The mice used in this experiment were accustomed to standard top-opening cages and when they first entered the experimental cage they were put in through the roof. So, until this point in their lives, the top of a cage represented a potential escape route. Although mice spent a lot of time interacting with the cage roof during the first session, this declined over time, with a particularly large reduction after the first session that coincided with their first experience of a side-opening cage. This suggests that the mice learnt that the escape route was no longer at the top of their cage.

Males and females spent a similar amount of time chewing at the external cage bars and there were no differences according to whether mice were singly housed or group-housed. However, group-housed males showed a difference in the relative amount of time spent near the internal and external bars, showing a weaker preference for the external bars than the other sex and group-size classes. This may have been attributable to aggressive competition within male groups, resulting in one dominant individual (Hayashi 1996) that may have controlled access to the area surrounding the escape route or was avoided by more subordinate males which therefore spent more time in other parts of the cage. However, although group-housed males showed a weaker bias in location near to the bars, this did not affect the bias in bar chewing, which was a much more specific measure of attempted escape behaviour.

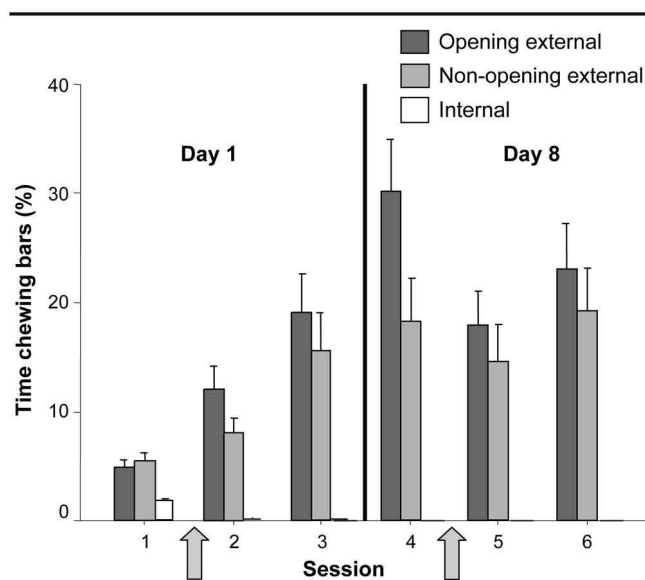
The preference for chewing external bars that opened regularly over non-opening external bars was initially weak after only one chance to escape, and even after seven days' experience of escape the mice continued to spend some time chewing at non-opening external bars. The continued interest in non-opening bars may have been because mice had difficulty in discriminating between the identical external bars once they were both closed. Alternatively, they may have been able to discriminate between the two types of bars, but tried all possible alternatives in attempting to seek a way out of their home cage. As the non-opening external bars were identical to those that occasionally opened and also appeared to provide a doorway to the outside, it would be appropriate to direct some attempts to escape towards this set of bars.

The increase in chewing at the external bars with increasing time since last escape may suggest that the motivation to leave the home cage dropped immediately after the animals had experienced escape but gradually increased as the mice were once more confined and unable to escape. Alternatively, the mice may have been anticipating the next escape opportunity, since these opportunities occurred at regular intervals.

Animal welfare implications

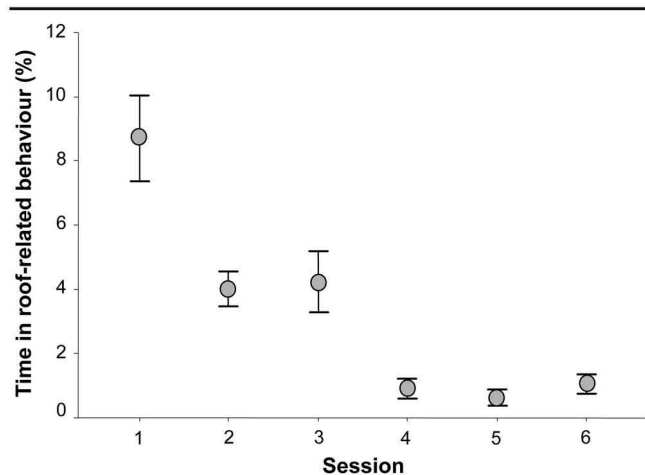
Since caged laboratory mice use bar chewing as a means to attempt escape from their home cage, it should be possible to use the levels of this behaviour to assess their motivation to escape from or leave different types of cage or housing condition as a comparative measure of the suitability of different environments from the animal's point of view. Thus, the desire to escape from a high-quality cage that provides a more acceptable environment should be less than from a poor-quality environment, which may be evident from the amount of effort put into, or time spent chewing at, external cage bars. The bar-chewing behaviour shown by mice in this study may have reflected a desire to avoid conditions within the home cage or an attempt to leave the home cage to merely explore a wider area or to seek a specific resource. If bar chewing was related to exploratory behaviour as opposed to avoidance of the home cage, then this would still

Figure 4



Percentage of time spent chewing at the three sets of bars according to session (mean \pm standard error per cage for all mice). Arrows indicate time of escape.

Figure 5

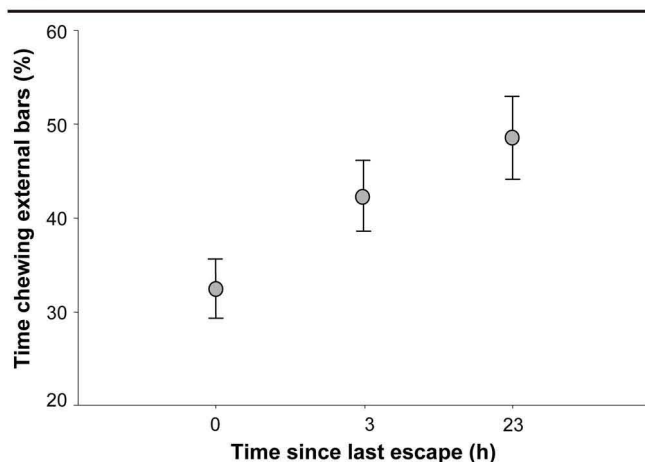


Percentage of time spent interacting with the roof according to session (mean \pm standard error per cage for all mice).

suggest that something was missing in the home cage of the animal. The mouse may not be motivated to avoid the home cage and to find a different environment but instead to seek a way out of the cage that would allow further exploration, in order to find the desired resource. This experiment could not differentiate between these two hypotheses. However, in both cases, bar chewing would suggest that something was inadequate or missing from the home cage, and so the measurement of escape behaviour could still be a valuable indicator of welfare. To test this, a comparison is required of bar chewing in different housing conditions that differ clearly in resources that are known to be desirable to mice.

A number of other potential escape-related behaviours, such as jumping up the cage walls (Ödberg 1986; Wurbel *et al* 1996) or digging (Wiedenmayer 1997), may also need to be

Figure 6



Percentage of time spent chewing at the external bars (opening and non-opening combined) on day 8 according to time since mice last experienced an opportunity to escape through external bars (mean \pm standard error per cage for all mice). Data are shown for sessions 5 (0 h), 6 (3 h) and 4 (23 h).

taken into account particularly when considering specific strains, and any method for measuring welfare would need to consider all of these different behaviours. Bar chewing appears to be common to most strains of mice and rats where they have access to external bars. It is important to recognise, however, that activity levels and behaviour can vary between strains (Barnett *et al* 1988; Nevison *et al* 1999b). Thus, while bar chewing by equivalent animals may be compared between housing conditions, it would not be appropriate to conclude that a strain that showed lower levels of escape-related behaviour than another strain was necessarily more content. However, an objective measure of escape-related behaviour may provide a useful internal comparison of different husbandry regimes and result in a practical opportunity to assess the response of animals to their home cage from a welfare perspective, particularly because not all animals will respond in the same way to the stress of a poor environment (Cooper & Nicol 1996). The bar-chewing behaviour shown by mice in this experiment was not stereotypic but this study involved only young adults (up to eight weeks old). Further work is required with older animals to assess whether bar chewing may eventually become stereotypic (Wurbel *et al* 1996) or, conversely, decline as animals learn that escape is not possible.

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