

There's no place like home: Cage odours and place preference in subordinate CD-1 male mice

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Running head: Soiled mouse bedding and place preference

Keywords: CD-1 mouse; social rank; conditioned place preference; odour cues

Abstract

Prior studies using mice have shown that scent marks are an important source of information and can cause behavioural changes in other individuals. Studies have also shown that scent marks in the environment can affect the outcome of social interactions between mice. We used conditioned place preference tests to investigate whether CD-1 male mice (*Mus musculus*) are reinforced by olfactory cues from the home cage. Soiled bedding from the home cage was presented in the initially less preferred chamber of the apparatus to determine whether this association would reduce the unconditioned preference for one chamber over the other. We tested the effects of social rank and housing condition by comparing the performance of dyads that were polarised into dominant and subordinate relationships, both when paired and when separated, with mice that were isolated throughout. The development of conditioned place preference (CPP) supported by home cage odours was influenced by social rank but not by housing condition. Only subordinate mice showed CPP to home cage odours, and this effect was seen irrespective of whether they were housed with a dominant cage mate or alone. Neither dominant (paired or separated) nor isolated mice showed any change in their preference for the chamber associated with home cage odours. This suggests that the smell of home is a more powerful reinforcer for subordinate mice in that it can produce contextual conditioning to the environment in which it is experienced.

Laboratory rodents spend a large amount of time in their home cage environment.

How do they like it? It is known that the home cage can act as an effective reinforcer for mice tested in a radial maze procedure (45). However, very few studies have investigated how mice rate the home cage environment relative to alternatives, but see (59,60).

An important aspect of the home environment is whether the animal is housed singly or in a group. In their natural state, house mice (*Mus domesticus*) typically live in social groups (24,25,26,38,48). As would be expected, what evidence there is suggests that group housing promotes welfare in laboratories (20,61,64,65).

Moreover, in the case of mice, there is good evidence that single housing of mice can result in behavioural and physiological abnormalities (22,36,37,63).

However, there is a cost to social housing in aggressive animals such as male mice which fight to establish a social hierarchy (8,49,52,53). There is good evidence that the stress response produced by social stimuli is even greater than that produced by stressors such as foot-shock or food or water deprivation (34). Nevertheless, in the laboratory, it has been found that male mice prefer social contact with a conspecific, compared to nesting material as an alternative form of environmental enrichment (65, but see 51).

The costs and benefits of social living are highly likely to depend on social rank within the group (10,19,27,57). The effects of subordination in laboratory rodents are diverse and include behavioural, and neurological alterations, as well as changes in the neuroendocrine and immune systems (43). Negative effects have also been observed in dominant male mice: hypertension (39), impaired sexual behaviour (11), increased locomotion, exploratory behaviour and elevated corticosterone (3), elevated

heart rate and hyperthermia (2), and reduced body weight and lack of corticosterone suppression (4).

Odour cues, provided by urine marks and glandular secretions, are very important to mice (9,15,24,31,50,67) and they produce up to 1 mg of protein per day for use in urine marking (1). Odour cues contain a wealth of information about the donor such as gender, sexual maturity, health, social dominance, even individual identity (6,28,29,30,62,64). In caged mice, urine marks and glandular secretions impregnate the bedding which therefore becomes secondarily reinforcing. For example, aggressive responses to intruder mice can be heightened when residents are surrounded by their own odours (32,33). Conversely, intruder males are less likely to attack a male whose odours match the surrounding substrate odours, and more likely to attack males whose odours do not match those on the surrounding substrate (16,18). This then has implications for best husbandry practice: laboratory cage cleaning can cause an increase in aggression between group-housed mice, presumably because it disrupts the odour cues within the cage (20,64). However, transferring odours between the soiled and clean cage, e.g., on nesting material, can reduce (64) or increase (20) aggression after cleaning.

The conditioned place preference (CPP) paradigm provides a convenient test of how cues can come to support contextual conditioning. This was originally developed to investigate the rewarding or aversive nature of drugs: the context in which the drug is experienced becomes secondarily rewarding or aversive and the animal chooses to spend time there or elsewhere. CPP has since been used to investigate the contextual conditioning supported by a wide range of stimuli produced by more naturalistic experimental manipulations, including maternal odours (21), sexual and aggressive behaviour (46), intermale aggression (44), tickling (7) and vaginal lavage (66).

Here we investigated whether exposure to home cage odours would support CPP. We also tested whether this would be influenced by social status and housing condition. If only pair-housed mice show CPP to home cage odours, this would be evidence that male mice can be positively reinforced by social housing conditions. If the odour cues in home cage bedding reduce aggression (20) then we would expect to see CPP in subordinate but not dominant males (44). Comparison between dominant and subordinate males that have been separated and re-housed as singletons with those that remain paired will tell us how long lasting any effects of social status may be (13).

Methods

Animals

Subjects were 34 male CD-1 mice aged 23 weeks (Charles River, UK), weighing 38 – 65g, fed on mouse standard diet (Harlan, UK). Mice were maintained on a reversed 12:12-h light / dark cycle (white lights on 20.30 – 08.30). Testing was carried out during the dark (active) phase, under dim (40W) red lighting. Animals were marked with black eyelash dye (Colorsport 30 Day Mascara, Brodie and Stone Plc, London, UK) to enable individual identification.

Housing

Mice were housed in standard opaque polypropylene laboratory cages (48 x 15 x 13cm; model M3, North Kent Plastics, UK). There were three housing conditions: isolated (single-caged throughout, n = 10), paired (males housed in dyads throughout n = 12) and separated mice (initially paired for two weeks to establish dominance ranks and then separated and singly-housed, n = 12). The separated housing group were used to test whether the effects of social housing and its concomitant stress

would persist after social interactions had been stopped. For the initial two weeks of pair-housing behavioural observations were made daily during 30 minute observation sessions. Behaviours were classified into aggressive, defensive, and submissive categories (42). The individual that made the most aggressive acts within each dyad was by definition dominant and the male that made the most submissive acts was subordinate in terms of their respective social rank (14). The frequency of submissive and aggressive acts was also used to match dyads to be separated with those that would remain paired.

CPP Apparatus

This was a 3 chamber mouse place preference box (model ENV-3013, Med Associates, Vermont, USA) with automatic guillotine doors and variable lighting. The apparatus was divided into three chambers. There were two choice chambers, one black and one white, connected by a smaller central grey chamber with a smooth floor (7.2 x 12.7 x 12.7cm). The white chamber had a stainless steel mesh floor and the black chamber a contrasting stainless steel grid rod floor (16.8 x 12.7 x 12.7cm). Under the floor of each chamber was a removable steel waste pan. Movement through the apparatus was recorded by photobeams, six in both of the choice chambers and two in the smaller central chamber, which relayed data to a PC (Viglen, Contender, P3 450). Lights were used in each chamber to aid mice in discriminating between the chambers (100mA, 80% intensity).

Procedure

The CPP procedure used here was based on the method used to test the CPP supported by aggressive interactions (44). Because of the number of mice in the study, the experiment was run in two replications over alternate days (fully counterbalanced for treatment). The apparatus was cleaned using diluted detergent

(Tego 2000, Goldschmidt Ltd, Buckinghamshire, UK) between each subject. Social odour cues were provided as clean or soiled sawdust bedding. The amount used, clean or soiled, was in each case set at 10g as this amount was readily accommodated in the waste pan.

Pre-conditioning: Mice received three pre-conditioning sessions during which they were placed individually in the central grey chamber with the doors to the alternative black and white chambers closed. After 1 minute the doors were raised and the mouse was allowed to explore the whole apparatus for 10 min, with no sawdust bedding present. On the third pre-conditioning session, the time spent in each of the choice chambers was recorded and used to determine the least and most preferred chamber for each mouse.

Conditioning: Mice received 8 conditioning sessions. On sessions 1, 3, 5, and 7, mice were confined individually in their least preferred chamber for 10 min with sawdust from their home cage in the waste pan underneath the floor. Home cage sawdust bedding was collected in the morning of each of these conditioning days before testing started. The sample was taken from throughout the home cage and care was taken to remove all faecal matter. On sessions 2, 4, 6, and 8, mice were confined in their most preferred chamber for 10 min with clean sawdust in the waste pan. In all conditioning sessions the number of fresh faecal boli deposited was recorded.

Post-conditioning: Mice received one post-conditioning test 48 hours after the final conditioning session. Each subject was placed individually in the central grey chamber with the doors to the black and white chambers closed. After 1 min the doors opened and the mouse was allowed free exploration throughout the whole apparatus for 10 min, with no sawdust present. Time spent in each of the choice chambers and in the central chamber was recorded for analysis.

Data analysis

All data was analysed using parametric tests in SPSS version 12.0.1 (SPSS Inc, Illinois, USA). The behavioural observations were analysed using analysis of variance (ANOVA) with social status (dominant or subordinate) and housing condition (paired or separated) as between-subjects factors. The isolated group were not included in this analysis as they were not housed in a social environment at any time during the study. To test for the development of CPP, repeated measures ANOVAs were used to analyse the difference in time spent in the least preferred chamber pre- and post-conditioning (before and after it had been paired with home cage odours). The between-subjects effects of housing condition and social status were examined separately because rank was not an issue for the isolated housing group. The colour of the least preferred chamber (black or white) was in each case included as a between subjects factor as chamber cues have been found to have an effect (44). The same analyses were then conducted on the times spent in the initially most preferred chamber, and in the central chamber (though in this case without the colour factor). Defecation scores (44) were also analysed in the same design to test for differences in the number of faecal boli produced on clean versus home cage bedding during conditioning. The procedure required that the clean bedding should always be placed in the most and the soiled bedding in the least preferred chamber, so bedding and chamber are inevitably confounded. For convenience, we refer to this factor as bedding. Overall body weights were compared at the start and end of testing.

Significant effects identified by ANOVA were further investigated using t-tests to compare groups, two-tailed unless otherwise stated. In the case of planned comparisons that were only a small subset of the possible comparisons, the inflation of familywise Type 1 error rate was not very large (23). However, to be conservative,

Bonferroni correction procedures were applied in the case of unplanned comparisons, adjusting the acceptable p value for the number of comparisons conducted.

Results

Behavioural observations

The number of aggressive, defensive and submissive acts observed during the 2-week social rank establishment period was compared for the pair-housed and separated groups. For these groups, we were also able to conduct analyses by social rank. These results showing the expected differences in aggression between mice of different social status are included in order to confirm that the social status allocations had been appropriate. For the number of aggressive acts, there was a significant effect of social status ($F(1,20) = 9.693$, $p = 0.005$). This arose because the mean (\pm standard error) number of aggressive acts made by dominants was 35.3 (± 10.38), compared with 1.4 (± 0.73) in subordinate groups. There was no effect of housing condition and no interaction between these factors ($F_s < 1$). Similarly, for the number of submissive acts there was also a significant effect of social status ($F(1,20) = 9.445$, $p = 0.006$). The mean (\pm standard error) number of submissive acts made by dominants was 0.4 (± 0.19), compared with 33.6 (± 10.31) by subordinates. By contrast, for the number of defensive behaviours, there was no effect of social status or housing condition and no interaction between these factors (maximum $F(1,20) = 1.957$).

Conditioned place preference

Least preferred chamber paired with home cage bedding

Housing condition: There was no significant effect of housing condition (isolated, paired or separated) either on its own, or in an interaction with chamber colour (maximum $F(2,28) = 1.004$). There was no overall difference in the time spent in the

least preferred chamber between pre- and post-conditioning test ($F(1,28) = 2.161$).

The only significant effect in this analysis was the colour of the least preferred chamber ($F(1,28) = 4.721$, $p = 0.038$). When the least preferred chamber was black the overall time (\pm standard error) spent in this chamber was 164.3 (± 6.69) s; when the least preferred chamber was white, the overall time spent in this chamber was 191.3 (± 10.46) s.

Social status: A second repeated measures ANOVA was used to analyse the effect of social status (dominant or subordinate) on the time spent in the least preferred chamber at the pre- and post-conditioning tests (before and after it had been paired with home cage odours). The two housing condition treatments where animals formed social relationships (paired or separated) were also included as a between subjects factor, and again colour of the chamber was also included.

This analysis showed a significant interaction between pre- and post-conditioning test and social status ($F(1,16) = 4.597$, $p = 0.048$). This means that the development of CPP depended on social rank. Subordinates showed the predicted effect: post-conditioning they spent significantly longer in the previously least preferred chamber ($t(11) = 1.90$, $p = 0.042$, one-tailed) after it had been paired with home cage odour. Dominants by contrast did not show CPP: there was no difference in the times spent in the chamber paired with home cage bedding ($t(11) = 0.34$), see Figure 1. No other factors or interactions were significant (maximum $F(1,16) = 4.323$). Thus on this analysis the overall time spent in the chambers was not influenced by the colour of the initially least preferred chamber.

---- Figure 1 about here ----

Most preferred chamber paired with clean bedding

Housing condition: There were no effects of housing condition either on its own, or in interaction with chamber colour ($F_s < 1$). There was an overall reduction in the time spent in this chamber post- relative to pre-conditioning ($F(1,28) = 7.384$, $p = 0.011$). The mean time spent in the initially most preferred chamber (\pm standard error) dropped from 252.1 (± 7.97) s to 223.4 (± 8.35) s after conditioning. However there were no interactions between the change in time spent in this chamber and any other factors (maximum $F(2,28) = 2.136$).

Social status: There were no effects of social status either on its own, in interaction with housing condition, or in interaction with chamber colour (all $F_s < 1$). Neither was there any change in the time spent in this chamber at the pre- and post-conditioning tests ($F(1,16) = 2.299$), nor any interaction between the change in time spent in this chamber and any other factor (maximum $F(1,16) = 2.669$).

Time spent in central chamber

Housing condition: A repeated measures ANOVA on the times spent in the grey central chamber at the pre- and post-conditioning tests show that housing condition had no effect on the overall time spent in this chamber ($F(2,31) = 1.365$). There was no change in time spent in this chamber between pre- and post-conditioning ($F(1,31) = 1.862$), neither was there any interaction between this repeated measures factor and housing condition ($F < 1$).

Social status: The isolated group was necessarily excluded from the sample, and a repeated measures ANOVA was performed with social status and housing condition (paired or separated) as factors. Social status had no effect on overall time spent in the grey central chamber ($F(1,20) = 1.489$), nor was there any interaction between social status and housing condition ($F < 1$). There was no change in the time spent in this chamber at pre- and post-conditioning, and no significant interaction involving this

repeated measures factor (all $F_s < 1$). Thus there was no indication whatsoever in the data that the central chamber provided an escape from differentially aversive bedding cues, see Table 1.

---- Table 1 about here ----

Defecation scores

Housing condition: There was a significant interaction between bedding and housing condition ($F(2,31) = 4.076$, $p = 0.027$). This was because whilst both isolated and pair housed mice produced significantly more faecal boli on clean compared with home cage bedding (minimum $t(9) = 4.34$, $p = 0.002$), separated mice defecated the same amount irrespective of the bedding that they were exposed to ($t(11) = 1.53$), see Figure 2.

---- Figure 2 about here ----

Social status: A second repeated measures ANOVA was used to analyse the effect of social status (dominant or subordinate) on the number of boli produced during conditioning sessions. The two housing condition treatments where animals formed social relationships (paired or separated) were also included as a between subjects factor. The isolated group was necessarily excluded from this analysis (see above). There was an interaction between bedding and social status ($F(1,20) = 5.833$, $p = 0.025$). This arose because whilst there was no significant difference in the amount of defecation on home versus clean bedding for dominants ($t(11) = 1.62$), subordinate mice defecated significantly more on clean compared with home bedding ($t(11) = 4.37$, $p = 0.001$), see Figure 3. There were no other effects of social status and no interaction between social status and housing condition (maximum $F(1,20) = 3.044$).

---- Figure 3 about here ----

Body weight

Housing condition: The different housing conditions resulted in different overall body weights ($F(2,31) = 3.862$, $p = 0.032$). Although there was a clear trend, mice in the paired housing condition were not significantly heavier than the isolated mice ($t(20) = 2.45$). There were no significant differences between the separated and the isolated group or between the separated and the paired group (maximum $t(20) = 1.66$). The mean body weights (\pm standard error) were as follows: for isolated mice, 41.0 (± 0.91) g; for paired mice, 46.1 (± 1.76) g; for separated mice, 43.1 (± 0.93) g. All groups gained weight during testing ($F(1,31) = 19.499$, $p < 0.001$). Whilst significant this effect was trivially small: overall body weights (\pm standard error) were 43.0 (± 0.88) g at the start of testing and 44.1 (± 0.77) g at the end of testing. Moreover this increase in weight did not interact with housing condition ($F < 1$), so housing did not affect the animals' ability to gain weight.

Social status: Other than the increase in weight ($F(1,20) = 12.519$, $p = 0.002$), reported for the full sample above, there were no significant main effects or interactions (maximum $F(1,20) = 2.116$).

Discussion

The development of CPP supported by home cage odours was influenced by social rank but not by housing condition. Only subordinate mice showed CPP to home cage odours, and this effect was seen irrespective of whether they were housed paired or separated. CPP in subordinates was demonstrated as a change in relative preference rather than a reversal in preference. As Figure 1 shows, the conditioning procedure increased the time that subordinates subsequently spent in the less preferred chamber (measured at the post-conditioning test). Figure 1 also shows that subordinates tended

to spend less time in the most preferred chamber after conditioning. After conditioning, all of the mice spent less time in the initially most preferred chamber, but unlike the increase in time spent in the least preferred chamber that was specific to subordinate males, statistically this decrease was not affected by social rank or housing condition. The non-significant tendency for subordinates to show a particular reduction in the time spent in the most preferred chamber could reflect some active aversion to the chamber associated with clean bedding, but the times spent in each of the chambers are of course likely to be inversely related. The mice have to be somewhere in the apparatus and there were no significant effects of social rank on the time spent in the central area.

Neither dominant (paired or separated) nor isolated mice showed any change in their preference for the chamber associated with home cage odours. This suggests that the smell of home is a more powerful reinforcer for subordinate mice in that it can produce contextual conditioning to the environment in which it is experienced. Since the home cage bedding could contain odours from a dominant conspecific, as well as own odour, and the effect was seen irrespective of housing condition, this result implies that even the presence of odours from a dominant conspecific does not prevent the bedding odour from acting as a reinforcer in this way. Similarly, bedding from cages containing dyads, rather than singletons, may have contained a higher concentration of odour cues. However, as there was no evidence in the results that CPP was affected by housing condition, we should conclude that the critical factor is the presence or absence of odour cues rather than their relative concentration.

The advantage of using a CPP procedure to investigate the rewarding or aversive properties of stimuli is that all tests are carried out in the absence of the stimuli. This is unlike traditional (unconditioned) preference procedures where the subject directly

interacts with the stimuli under test (58,59,65). By definition, CPP tests the level of secondary reinforcement that accrues to the previously neutral cues provided by the apparatus. Thus our results show that subordinate male mice' preference for soiled home cage bedding is strong enough to alter the unconditioned preference for a distinctive environmental context by this associative process.

The role of other chamber cues

CPP was demonstrated by showing that, dependent on social rank, associations with soiled bedding rendered the previously least preferred experimental chamber a relatively more attractive place to spend time. The two chambers of the apparatus were distinctively different, in colour and floor type and original chamber preference was different for different mice. Accordingly we also conducted analyses to see whether the colour of the conditioning chamber made a difference to the development of CPP, as has been found in previous work (44). There was some evidence for an effect of colour of least preferred chamber on the overall time spent there, seen on the analysis by housing condition. However, this effect was not reliable in that it did not show on the analysis by social rank that was necessarily conducted with a smaller sample size as this latter analysis excluded isolates. Moreover, there was no evidence whatsoever that the colour of the least preferred chamber moderated the development of CPP.

The conditioning procedure adopted meant that bedding and chamber were inevitably confounded. Thus the fact that the identity of the chamber initially least preferred made no difference to the observed results suggests that it was the properties of the conditioning cues (clean versus soiled bedding) that were critical for establishing CPP, and producing different levels of defecation (see below), rather than the

environmental cues (chamber colour and floor type) that can also moderate the level of conditioning depending on the UCS in use (44).

Defecation on novel versus familiar bedding

Differences in the amount of defecation as a measure of emotionality (44) in the different chambers suggested that bedding and other contextual cues provided therein were salient. How the mice were housed made a difference to the level of defecation: consistent with heightened emotionality, isolated mice defecated the most overall.

There were also some differences in the amount of defecation on clean and soiled bedding. Isolated and pair-housed mice produced more faecal boli on clean compared to home cage bedding (mice in the separated housing group were indiscriminate).

This difference in isolated mice presumably reflects heightened sensitivity to environmental change. In pair-housed and separated mice differences in defecation depended on rank.

This rank effect took the form that subordinates produced more faecal boli during conditioning sessions with clean sawdust (in the most preferred chamber) compared to sessions with home cage sawdust (in the least preferred chamber). These effects were seen irrespective of whether the mice were housed separately or in pairs. Thus although differences in defecation depended on social rank, they were not influenced by the presence or absence of other mouse odour. This finding is consistent with earlier reports of reduced or inhibited scent marking in subordinates, although these studies measured urine production rather than defecation (12,41). By contrast, dominants showed no difference in their level of defecation on the clean and soiled bedding. This pattern of effects may also suggest that subordinates were more anxious in the environment with the clean sawdust. Since aggression is known to be increased by home cage cleaning (20,64) one possibility is that, through normal husbandry

practices, clean sawdust had earlier become associated with an increased severity or frequency of aggressive interactions. By definition, these encounters particularly impact on subordinate mice which suffer defeat by dominants (5,43,44,54,56).

The role of odour cues

It is known that there are differences between dominant and subordinate mice in how they approach and investigate odours from other males (17,24,26) and their preferences for odours from different donors (17,55). During conditioning, subordinates may have found it more rewarding to investigate the odours from the home cage in the least preferred chamber compared with exploring the odourless bedding in the preferred chamber. As discussed above, the normal routine of animal husbandry could well have the consequence that soiled bedding became associated with a decreased frequency of aggressive interactions, and thus differentially rewarding for subordinates.

Conversely, as dominant mice frequently countermark other male's marks (26), dominants may have found it unrewarding to spend time in areas already marked with their scent. Dominants did not show any change in their relative preference for either chamber, whether paired with home cage or clean bedding, suggesting that bedding cues have little or no salience for dominant mice. As single-housing is suggested to stimulate dominance in male mice (36,40,63), this may be the reason that isolated mice, like dominants, failed to show a CPP.

Separated subordinate mice also showed a CPP to the chamber paired with home cage odours. Thus if the reinforcing capacity of the bedding was acquired earlier, this effect must be long-lasting. Mice in the separated housing condition experienced an initial two week period of pair housing and were then re-housed singly for 11 weeks until the start of CPP testing. In rats, effects of social stress are known to persist for

over two months after the last social defeat (47). Moreover, sensitisation to stressful stimuli develops during the weeks after the initial social defeat (35). The development of a CPP by separated subordinate mice in this study shows that a brief period of social stress had long term consequences for these mice, which were not reversed by stopping social interactions and re-housing them singly. We have previously observed a similarly long-lasting effect in subordinate CD-1 males in tests of spatial alternation on the T-maze (13).

We propose that the increase in time spent by subordinates in the less preferred chamber, demonstrated after this chamber was paired with home cage odours, reflects the fact that home cage odours are rewarding for subordinates. Logically, it is possible that they spent more time in this environment simply because they were avoiding the chamber associated with clean bedding. However, our analyses show that only subordinates increased their preference for the chamber paired with home cage odours. Statistically, all mice showed some reduction in time spent in the initially most preferred chamber after it had been paired with clean bedding. Moreover, as discussed above, with naturalistic cues of the kind used in the present study it can be difficult if not impossible to find a truly neutral comparison cue.

Conclusions and implications

In conclusion, our results show that subordinate male mice found home cage sawdust rewarding as evidenced by a CPP to this stimulus. The CPP in subordinate mice could have been determined by the rewarding effects of the familiar odours in the home cage sawdust, an aversion to the lack of odours in the clean sawdust, or a combination of these factors. Separately housed subordinate male mice also showed a CPP towards home cage odours and thus a brief period of social stress had a long term consequence for these mice which was not reversed by stopping the social interactions and re-

housing these mice as singletons. Thus irrespective of how the effect was mediated, the perception of home cage odours differed between mice of differing social rank and that these differences persisted after the exposure to social stress had ended.

Finally, it is also possible that social rank will affect sensitivity to other reinforcers that will therefore support CPP under conditions in which CPP is not demonstrable in cage mates of the alternate social status. We would predict that such changes in the effective salience of cues should be particularly likely with naturalistic reinforcement, such as that provided by feeding, sexual or aggressive behaviour, because differences in life history strategy will result in different trade-offs between fitness components.

Acknowledgements

We thank Andy Smith for technical support. A.F. is supported by a BBSRC Committee Studentship.

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Table 1

Group	Pre-conditioning	Post-conditioning
<i>Isolated</i>	177.9 (8.78)	202.7 (16.73)
<i>Paired</i>	166.1 (17.68)	175.0 (11.38)
<i>Separated</i>	198.0 (21.78)	205.2 (15.73)
<i>Dominant</i>	197.3 (22.08)	200.7 (16.71)
<i>Subordinate</i>	166.8 (17.42)	179.5 (10.90)

Table 1: Mean times spent in the grey central chamber at the pre- and post-conditioning tests, shown separately by housing condition and social status. Numbers in brackets are the standard error (\pm) about the mean.

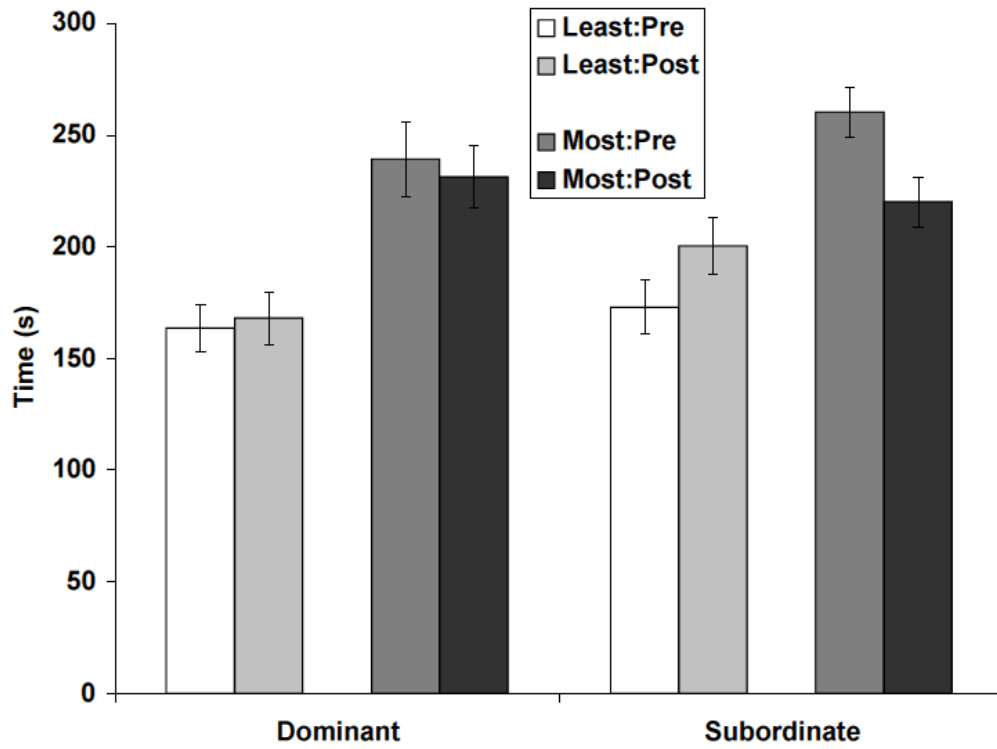


Figure 1: Time spent in the least preferred and most preferred chambers at pre- and post-conditioning tests for mice of different social status (dominant or subordinate).

Error bars are the standard errors about the mean.

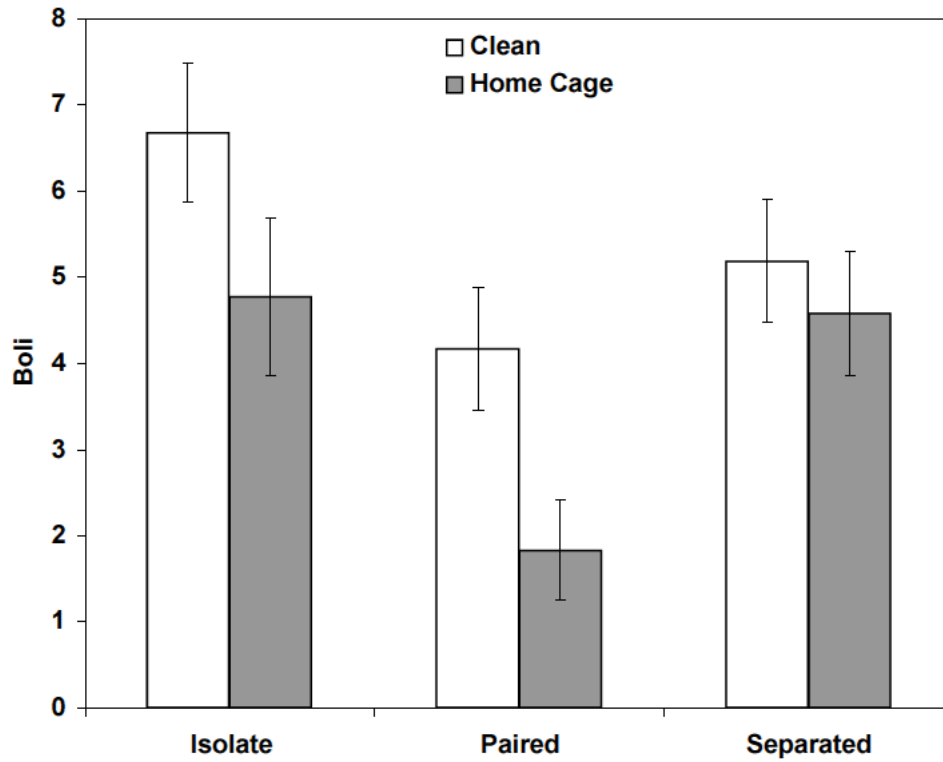


Figure 2: Defecation on home cage versus clean bedding during conditioning depends on housing condition (isolated, paired or separated). Error bars are the standard errors about the mean.

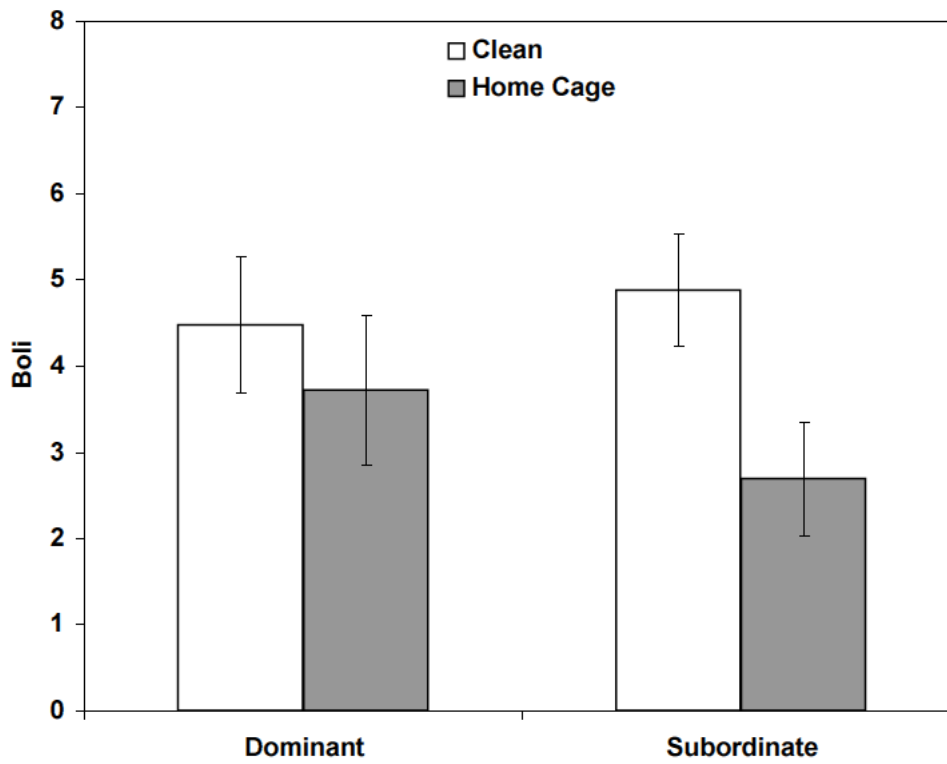


Figure 3: Defecation on home cage versus clean bedding during conditioning depends on social status (dominant or subordinate). Error bars are the standard errors about the mean.