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Disruptive effects of standard husbandry practice on laboratory rat social discrimination

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Abstract

Elements of husbandry procedures, such as handling, may disrupt rodent social behaviour. Such effects may be contingent upon the familiarity between individuals and upon the quality and quantity of the disruption. We investigated this issue using laboratory rats. We placed 36 rats into groups of three. At the point of group formation, and at 24 h, 7 days and two weeks afterwards, individuals received one of three treatments: 'handling', exposure to novel conspecific 'urine', or 'control' (undisturbed), for a duration of either 5 or 15 mins. We used a social recognition test to measure the ability of the rats to recognise the urine of group members of increasing familiarity following the implementation of these treatments. The 'control' treatment did not appear to disrupt social recognition. The 5 min 'urine' treatment appeared to disrupt recognition only when the rats had received the briefest experience of the 'familiar' urine (5 mins). The 5 min 'handling' treatment, however, appeared far more disruptive, with an apparent disruption of social recognition even when familiarity with the urine donor was high (eg 7 days of group housing). Both the 'handling' and 'urine' treatments appeared more disruptive when presented for an increased duration (15 mins). There was also some evidence that increased experience of the handling procedure might reduce its disruptive effect. The results of this study have several implications for the welfare of laboratory-housed rats, and these are discussed.

Keywords: *animal welfare, husbandry procedures, laboratory rat, social discrimination, social disruption, social recognition*

Introduction

Well-supervised group housing is now the recommended housing system for laboratory rodents (eg Rodent Refinement Working Party 1998). Rodents are most commonly housed in single sex groups, and, following initial mixing into that group, usually remain within the same social group for the rest of their lives. Following group establishment, dominance relationships are formed and the occurrence of potentially costly aggression is reduced (eg Hurst *et al* 1996). However, throughout this period, individuals may be removed temporarily for husbandry events, such as receiving an injection or cage cleaning, and then returned to their home cage. There is increasing evidence that the welfare of individuals in stable social groups may be compromised by these elements of standard husbandry practice, and with rodents being the most commonly used research animals this has potentially important implications for animal welfare. A better understanding of such effects will enable them to be minimised.

Within a laboratory setting, rodents are exposed to numerous different environmental factors as determined by the particular husbandry regime employed, and some of these factors appear to be capable of influencing rodent behaviour and may potentially compromise welfare. These include elements of: the background environment, eg ultrasound emitted by electronic equipment (eg Sales 1991); the housing environment, eg the housing of test animals close to controls (eg Beynen 1992); the human environment, eg handling during cage cleaning (eg Gray & Hurst 1995); and the olfactory environment, eg disruption of olfactory cues during cage cleaning (eg Van Loo *et al* 2000).

Some of these elements of husbandry practice appear to act directly on the social behaviour of group-housed rodents, resulting in an increase in aggression (eg Gray & Hurst 1995; Van Loo *et al* 2000). A possible explanation for this increase is that the ability of individuals to recognise previously familiar conspecifics has been disrupted as a direct result of the husbandry practice. This could be due to physical changes in the environment; for instance, a change in bedding substrate might disrupt olfactory communication by removing urine marks required for maintaining social tolerance (Gray & Hurst 1995) or might alter contextual cues (cf Burman & Mendl 1999). An alternative hypothesis is that in some instances there may have been a cognitive change in the animal itself, ie its memory has been disrupted in some way. As a consequence, re-acquaintance and the renewal of dominance relationships may be necessary following reintroduction (eg Ewbank & Meese 1971), resulting in the reappearance of inappropriate social aggression.

Research has demonstrated that stressful events can have a disruptive effect on animal cognition (eg de Quervain *et al*

1998; Mendl 1999). In a previous study (Burman & Mendl 2000) we observed an apparent disruptive effect of handling and of introduction to a novel conspecific on measures of short-term social memory in laboratory rats. If rats were handled for 5 mins following an initial introduction to an unfamiliar juvenile conspecific, the introduced juvenile did not appear to be recognised when reintroduced 15 mins later — a time after which recognition usually occurs (eg Dantzer *et al* 1987). Similarly, exposure to a novel juvenile conspecific midway between two exposures to a different juvenile also appeared to prevent recognition.

In that study the individuals to be remembered by the subject animal had been encountered for only a 5 min period. However, most group-housed laboratory rodents are kept together for long periods of time: weeks, months or even years. It is therefore important to examine whether apparent disruptive effects on recognition memory are also observed when the cue to be remembered becomes increasingly familiar. One might expect social memory to become increasingly resistant to disruption after longer-term memory formation because there has been greater opportunity for memory trace consolidation. In contrast, one might expect a memory trace that initially appears resistant to interference from a particular brief event (eg handling) to have a greater chance of being disrupted if the duration of the event is increased.

If common husbandry procedures such as handling can have a disruptive effect on social memory function, this will have a negative impact on welfare. The unnecessary increase in aggression and social investigation associated with the disruption of social behaviour will be costly both in terms of energy loss and in the increased potential for injury. Socially induced stress can also be a major cause of physiological and physical changes (eg blood pressure, immunosuppression and related kidney pathology), which may threaten an animal's welfare (eg Fokkema *et al* 1995; Hurst *et al* 1996). In addition to effects on welfare, there may be further consequences for the validity of data obtained from studies involving these animals. Individual differences between rodents, and differences between experiments, may be exaggerated in conditions where socially induced stress is enhanced. Elements of routine husbandry practice that have already been demonstrated to influence subsequent subject behaviour include handling, which appears to affect behaviour in anxiety tests (eg Lapin 1995; Schmitt & Hiemke 1998), and housing conditions, which can impose constraints on behaviour and brain development (Würbel 2001).

We therefore designed the current study to extend our previous research and to investigate whether increasing the length of time that animals have been housed together can affect the extent to which recognition of their group mates' urinary odours can be disrupted by selected common husbandry/experimental events of varying duration.

General methods

Subjects, housing and care

We used 36 conventional male Lister Hooded rats (Harlan, Bicester, UK). The rats were 2 months old at the start of the study and were housed in groups of three in standard laboratory cages (33 cm × 50 cm × 23 cm high; RC2/F North Kent Plastics) with sawdust litter, shredded paper for bedding and *ad libitum* food (Harlan Teklad Laboratory Diet) and water. We housed the rats in the same room in which they were to be tested, in a controlled environment (20 ± 1°C, 46% relative humidity), on a reversed lighting schedule (lights off 0800–2000 h) with dim white light (10 W) allowing some visibility both for the researcher and the subjects.

The rats were housed in a single room, with the different groups of three rats kept in separate racks and in different positions, ensuring at least 1 m distance between neighbouring groups to reduce familiarity between groups. When cages were cleaned, care was taken to ensure that rats did not come into contact with the odour cues of rats from different groups. Thus, while there may have been some familiarity with the airborne odours of individuals from different groups, rats were never able to directly investigate the urinary odours of individuals from the other social groups. Observations were made during the dark phase of the cycle in order to ensure that the rats were at their most active when the experiments were carried out. Disposable gloves (MAPA Professional) were worn when handling rats to restrict odour transfer. All of the rats were accustomed to brief periods of handling during cage cleaning.

Ethical considerations

Because of the possibility of aggression following the reintroduction of rats to their social groups, this research was carried out under a UK Home Office project licence (PPL 30/1470). A researcher was always present for at least 30 mins following either the allocation of rats to new groups or the reintroduction of rats to familiar groups after handling or exposure to the odour of a novel conspecific. This ensured that if there was any overtly aggressive (ie potentially injurious) behaviour, then the individuals concerned could be separated immediately. At no point in this study was injury caused by aggressive behaviour, and there was no evidence of long-term effects on the rats as a consequence of the experimental procedure — normal behaviour and weight gain were observed throughout.

Treatment groups and the social discrimination procedure

We used the social discrimination procedure (Engelmann *et al* 1995). This involved: 1) the introduction of a urine sample 'A' from a novel conspecific to a subject rat for 5 mins (the 'familiarisation encounter'); 2) a 15 min interval (the inter-exposure interval, 'IEI'); and then 3) the simultaneous introduction of the original urine sample 'A' and a novel conspecific urine sample 'B' for 5 mins (the 'discrimination test'). In this type of test, a significant preference to investigate the novel urine sample 'B' over the familiar urine sample 'A' is taken to demonstrate recognition of the familiar sample (eg Engelmann *et al* 1995). In contrast, no such preference is taken to suggest that the familiar sample 'A' has not been recognised.

We were interested in whether particular treatments would interfere with recognition over a time period after which

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recognition would normally occur (eg Engelmann *et al* 1995; Dluzen *et al* 1998, 2000; Reijmers *et al* 2001). During the initial IEI (ie before the first discrimination test), we imposed treatments either by handling the subjects ('handling' treatment) or exposing them to a previously unencountered urine sample from an unfamiliar (non cage-mate) conspecific ('urine' treatment), for a period of either 5 or 15 mins. 'Handling' consisted of the subject rat being picked up and held without restraint 30 cm above the ground for a period of 5 s every 15 s, either for 5 or 15 mins. For the 'urine' treatment, rats were introduced to 20 cm^3 of urine-soiled paper obtained from an unfamiliar conspecific (see below), either for 5 or 15 mins. As a 'control', rats were left undisturbed in their social group during the IEI either for 5 or 15 mins. There was therefore only a nominal difference between the 5 and 15 min 'control' treatments, with both treatments essentially being the same. Conditions were standardised for all three treatments, ie they were carried out by the same person in exactly the same way for each of the animals tested.Since the animals were group-housed and could not be tested in their own home cage without interference from cage-mates, testing, exposure to the urine of a novel conspecific, and handling all took place while the subject animal was in a test cage — an empty home cage with clean sawdust bedding. The test cage and its lid were disinfected (Virkon, Antec International) before testing each new subject, and the sawdust bedding was changed following each test with the same subject.

Urine samples

Urine samples consisted of urine-soaked absorbent tissue paper (BIP, Bristol Industrial Protection) presented in spherical stainless steel wire mesh containers (total volume 20 cm^3, diameter 3.7 cm) secured halfway up the wall of the test cage. The urine was collected by housing the donor rats individually on a clean plastic-floored cage for 1 h prior to the start of testing, with absorbent paper being used to collect the urine deposits. All urine samples were used within 4 h of collection and were stored in plastic 'cling-wrap' prior to use. The urine samples used for each batch of tests were collected at the same time and therefore were all of the same age. We returned the donor rats to their social group following urine collection and left them undisturbed for a further hour before testing. The wire mesh containers were changed and disinfected between each presentation to the subject rats to prevent odour deposition by the subject rats.

Behavioural observations

During the initial presentation to the subject rat, a single urine sample was placed centrally at one end of the test cage (16.5 cm from either side of the cage). For the discrimination test, one of the urine samples was placed centrally on the left of the test cage and the other on the right (both were 25 cm from either end). The positioning of the two urine samples was balanced across treatments to control for potential side preferences.

The subject's investigation of the urine samples was recorded directly using an event recorder (Psion Organiser II) with Noldus Observer software (Noldus Information Technology 1993), and with the researcher observing remotely by video camera to avoid influencing the subject's behaviour. Investigation included sniffing, licking and/or the subject's nose being held within 1 cm of a urine sample container.

Data analysis

At each test session (ie 5 min, 24 h, 7 days and 2 weeks after group formation, see below), we used paired *t*-tests (two-tailed) to determine whether subjects in the three different treatments were able to distinguish between samples 'A' and 'B' in the discrimination test. The total time spent investigating the novel and familiar urine samples was analysed using the Minitab statistical package (Minitab 1996). Unless otherwise stated, data met the assumptions of parametric statistical tests without requiring transformation. Data are presented below as non-transformed means ± standard errors.

Experiment 1

In this experiment we investigated how increasing social familiarity through group housing would affect the ability of three different 5 min treatments ('control', 'handling' and 'urine') to disrupt social memory.

Experimental procedure

In 'Test Session 1', the 5 min treatments were imposed after a 5 min 'familiarisation' encounter with the urine sample of a conspecific (see Figure 1a); for 'Test Session 2', treatments were imposed after 24 h of group housing (see Figure 1b); and for 'Test Session 3', after 7 days of group housing (see Figure 1c). In addition to the period of group housing (either 24 h or 7 days), rats in Test Session 2 and Test Session 3 were exposed to an additional 5 mins with the urine sample of a familiar (cage-mate) conspecific (designated sample 'A') (see Figure 1b & 1c) during the familiarisation encounter. We tested each of the 36 subject rats on one occasion in each test session, such that, by the end of the experiment, each rat had received all three treatments, one in each test session, with treatment order being determined by Latin square design (eg Sokal & Rohlf 1995).

Results

Test Session 1: after 5 mins of previous experience with the urine donor

Paired *t*-tests revealed that rats in the 'control' treatment ($t_{12} = -2.99$; $P < 0.05$), unlike those in the 'handling' and 'urine' treatments (both $P > 0.1$), showed a preference for the novel urine sample in the discrimination test (see Figure 2a). This suggests that 5 mins experience either of the 'handling' or the 'urine' treatments may have prevented the demonstration of discrimination between the urine samples.

Test Session 2: after 24 h of previous experience with the urine donor

Analysis of investigation in the discrimination test revealed that rats in the 'control' and the 'urine' treatments preferred to investigate the novel urine sample to the familiar sample ($t_{12} = -2.66$; $P < 0.05$ and $t_{12} = -2.51$; $P < 0.05$ respectively). However, rats in the 'handling' treatment showed no

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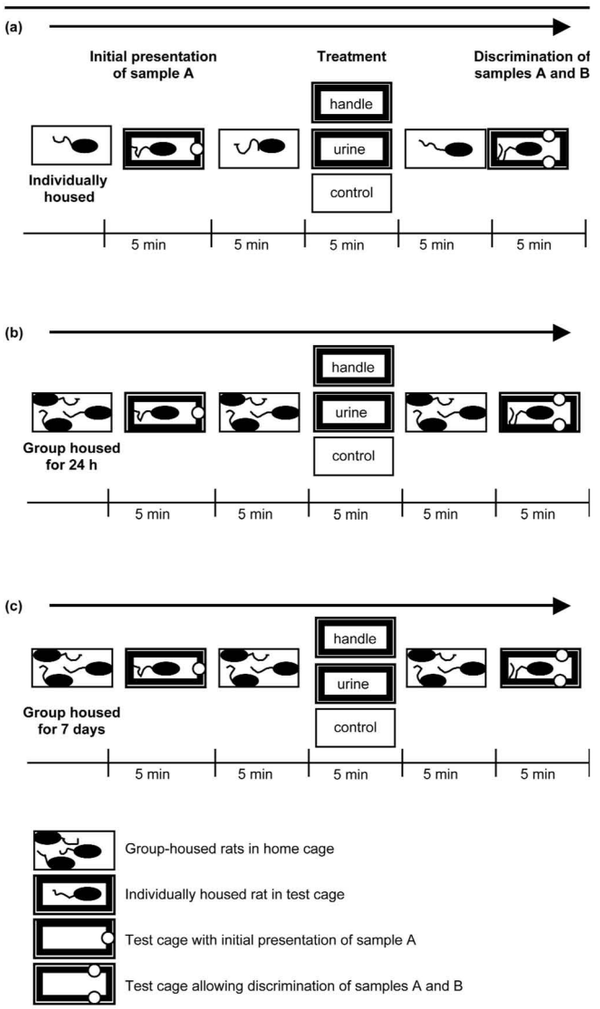
Figure 1



(a) Procedure for Test Session 1 (all treatments), with 5 min treatments and a 5 min discrimination test. The rats are initially individually housed but are group-housed at the end of the session. (b) Procedure for Test Session 2 (all treatments), with 5 min treatments and a 5 min discrimination test. The rats have been group-housed for 24 h prior to testing. (c) Procedure for Test Session 3 (all treatments), with 5 min treatments and a 5 min discrimination test. The rats have been group-housed for 7 days prior to testing.

such preference ($P > 0.1$; see Figure 2b). Thus it appears that when prior familiarisation with the donor of sample 'A' is increased from 5 mins to 24 h, discrimination between the two urine samples becomes possible after the 'urine' but not after the 'handling' treatment.

Test Session 3: after 7 days of previous experience with the urine donor

As observed in Test Session 2, whilst rats in the 'control' and 'urine' treatments showed a preference for the novel sample in the discrimination test ($t_{12} = -3.00$; $P < 0.05$ and $t_{12} = -2.91$; $P < 0.05$ respectively), rats in the 'handling' treatment showed no such preference ($P > 0.1$; see Figure 2c).

Experiment 2

The findings of Experiment 1 suggested there to be a link between the length of familiarisation with a conspecific and the susceptibility of a rat's social memory to subsequent disturbance. We therefore decided to investigate this further by looking at how increasing the duration of the disturbance treatment might interact with increasing familiarisation with a conspecific.

Experimental procedure

In this experiment there were only two test sessions: the first, 'Test Session 4', after the animals had been group-housed for 7 days, and the second, 'Test Session 5', after they had been group-housed for 2 weeks. In Test Session 4, both the 'control' and the 'urine' treatments were presented for 15 mins because neither treatment appeared effective at disrupting discrimination when presented for only 5 mins in Experiment 1. The 'handling' treatment was presented for 5 mins, since this had been adequate to disrupt performance in the discrimination task in the previous experiment. In Test Session 5, all three treatments were implemented for 15 mins. To minimise animal use, the rats used in Experiment 1 were used again in Experiment 2, having been arranged into new groups of three. Each rat was tested on two occasions, receiving two of the three treatments balanced for order according to a Latin square design (Sokal & Rohlf 1995), with care being taken to ensure that rats never encountered the odour of previous cage mates.

Results

Test Session 4: after 7 days of previous experience with the urine donor

Paired *t*-tests revealed that rats in the 'control' treatment showed a clear tendency to investigate the novel urine sample in preference to the familiar sample in the discrimination test ($t_8 = -2.18$; $P = 0.066$). Although this trend was not quite statistically significant, as one would have predicted, it is likely that this result reflects an underlying recognition of the familiar urine sample. Following 5 mins of handling, rats also showed a preference for the novel urine sample ($t_8 = -2.88$; $P < 0.05$). A possible explanation for this result is that because the rats had already received 5 mins of handling in the previous experiment their increased experience of handling had reduced the disruptive potential of this treatment. This hypothesis is supported by anecdotal observations of the rats' behaviour during the handling treatment, since the rats appeared to habituate to the handling procedure. In contrast, rats in the 15 min 'urine' treatment showed no preference for the novel over the familiar urine sample ($P > 0.1$; see Figure 3a). This suggests that increasing the duration of the 'urine' treatment from 5 to 15 mins may prevent the discrimination of the familiar urine after a 7 day familiarisation period.

Test Session 5: after 2 weeks of previous experience with the urine donor

Rats in the 'control' treatment again showed a strong tendency to investigate the novel rather than the familiar urine

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sample in the discrimination test (t_8 = –2.14; P = 0.069). Rats that had received the 15 min 'urine' treatment also showed a preference for the novel urine sample (t_8 = –2.6; $P < 0.05$), suggesting that if the period of group housing (familiarisation) was increased from 7 days to 2 weeks, even the increased 15 min 'urine' treatment no longer appeared to prevent recognition. However, those rats that had received 15 mins of handling showed no preference for either sample ($P > 0.1$; see Figure 3b). It therefore appears that the increased duration of handling overcame the effect of the increased experience observed in Test Session 4, resulting once more in an apparent failure to show a discrimination.

Discussion

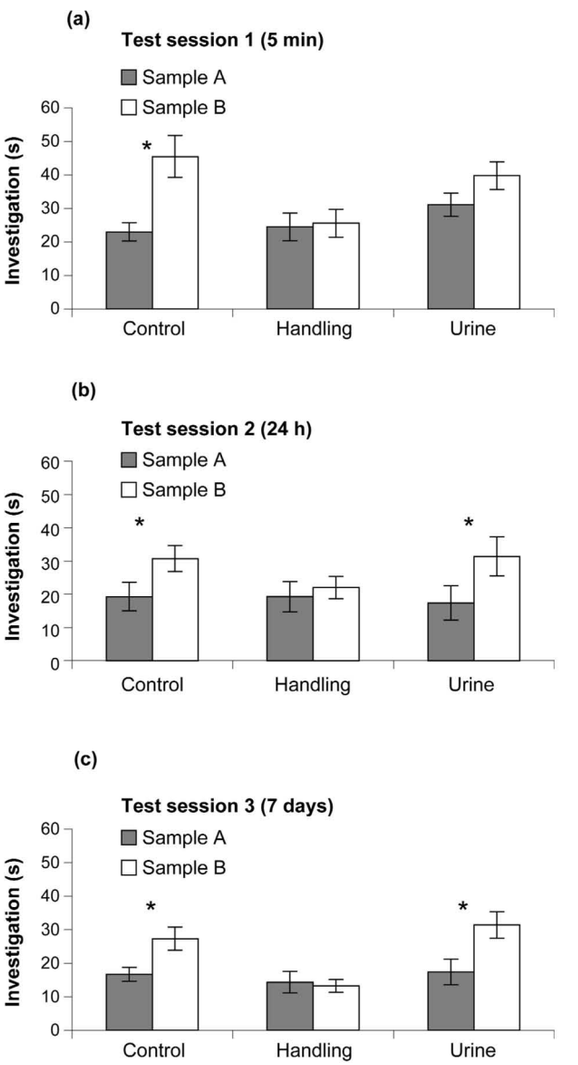
The results of this study suggest that, as previously predicted (Burman & Mendl 2000), if an individual becomes more familiar (spends more time) with the donor of a urine sample, then its ability to demonstrate discrimination of this urine becomes less vulnerable to interference by exposure to the urine of a novel conspecific. However, handling appeared to retain its ability to interfere with the demonstration of social odour recognition; hence, it seems to be a more potent disrupter of performance in the memory task studied here.

It also seemed that increasing the duration of time for which the treatments were experienced was effective in disrupting the recognition of urine samples that had, at that same level of familiarity, previously been recognised. Moreover, extending this level of familiarity further then appears to counter that increase in treatment duration. These results (summarised in Table 1) extend the findings of our previous study (Burman & Mendl 2000, see Introduction) and provide further evidence that olfactory cues may be sufficient for the recognition and/or the discrimination of conspecific identity (eg Sawyer *et al* 1984; Popik & van Ree 1998).

The results suggest that rats' memories of social odours may be vulnerable to retroactive interference, ie the ability of an interpolated experience to reduce performance on an original task upon subsequent testing (eg Rodriguez *et al* 1993). This disruption appears to occur whether the interfering stimulus/treatment is specific to the original task (ie the treatment is the same as the original task), eg exposure to the urine of an unfamiliar conspecific, or non-specific (ie the treatment is unrelated to the original task), eg handling. As with our earlier research (Burman & Mendl 2000), this result is contrary to previous suggestions that non-specific stimuli may not result in retroactive interference (Popik & van Ree 1998).

Disruption by a specific stimulus, ie by exposure to the urinary odour of an unfamiliar conspecific, suggests direct competition with the previous memory trace in a capacity-limited short-term memory capable of storing information about only one individual at a time (Squire 1986). Such disruption might be expected if the memory trace was acquired over only a 5 min period some 5 mins previously, but, following 24 h of housing or more, the additional time available for the consolidation of the memory trace may mean that the memory for the original (familiar) individual has shifted from short to long-term memory storage (cf Squire 1987). Thus, when the urinary odour of an unfamiliar individual is introduced following at least 24 h of group housing, space could have become available in the short-term memory store for the new memory trace to be consolidated, and therefore no apparent disruption of memory occurs. The ease with which a stimulus is remembered and its subsequent susceptibility to disruption may also be influenced by stimulus salience (eg MacPhail 1986). If it only becomes cost-effective to remember a newly encountered individual once it becomes clear that the individual is worth remembering, ie has future 'value' (because the maintenance of

Figure 2



Total amount of investigation in the discrimination test directed towards samples 'A' and 'B' for the three treatments: 'control', 'handling' and 'urine' experienced for 5 mins. (a) Test Session 1: 5 mins previous experience with urine donor. (b) Test Session 2: 24 h previous experience with urine donor. (c) Test Session 3: 7 days previous experience with urine donor. (* indicates a significant [$P < 0.05$] preference for the novel urine sample 'B'.)

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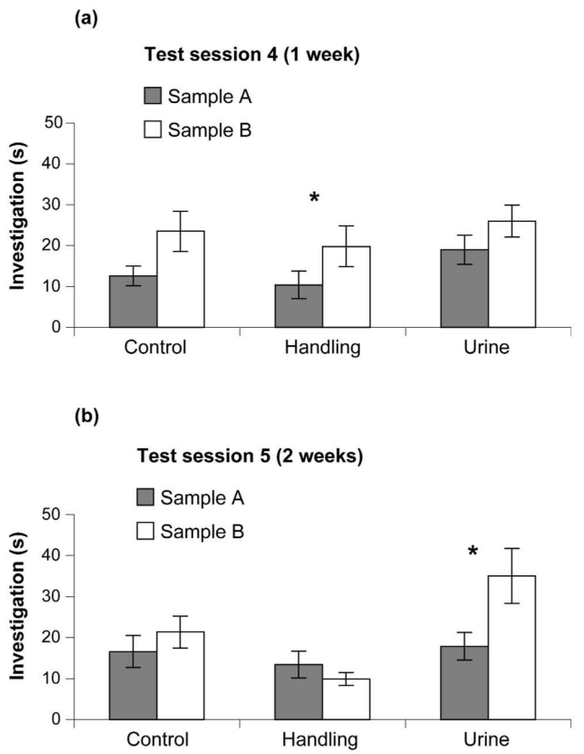
Table 1 Ability of rats to discriminate between a urine sample with which they were increasingly familiar and a novel urine sample, following the implementation of 'control', 'urine' and 'handling' treatments for varying lengths of time.

	Amount of previous experience with the familiar odour			
Treatment	**5 mins**	**24 h**	**7 days**	**2 weeks**
Control	Y	Y	Y/(Y)	(Y)
Novel urine (5 mins)	N	Y	Y	–
Novel urine (15 mins)	–	–	N	Y
Handling (5 mins)	N	N	N/Y	–
Handling (15 mins)	–	–	–	N

Y = discrimination, ie a significant preference for the novel sample; N = no discrimination, ie an apparent disruption caused by the treatment; (Y) = discrimination approaches significance; dash = treatment not tested at this time interval.

Figure 3



Total amount of investigation in the discrimination test directed towards samples 'A' and 'B' for the three treatments: 'control', 'handling' and 'urine'. (a) Test Session 4: 7 days previous experience with urine donor; 'control' and 'urine' treatments were 15 mins and 'handling' treatments 5 mins. (b) Test Session 5: 2 weeks previous experience with urine donor; all treatments were 15 mins. (* indicates a significant [$P < 0.05$] preference for the novel urine sample 'B'.)

memories may be costly [Dukas 1998]), then it could be that the 24 h period was sufficient for the identity of the groupmate to be considered 'valuable', and thus resistant to disruption from exposure to the urine of an unfamiliar conspecific.

However, this hypothesis does not explain the apparent disruption of recognition in Test Session 4 when exposure to the 'urine' treatment was increased from 5 to 15 mins. At this point the rats had been group-housed for 7 days, so why should a further 10 mins exposure to the urine of an unfamiliar conspecific prevent the demonstration of discrimination between the familiar and novel urine samples? It seems unlikely that any additional information could be derived from 15 mins exposure to the urine sample of an unfamiliar individual than could have been as easily gained from 5 mins exposure. It may therefore be the case that some other aspect of the 15 min 'urine' treatment, other than direct access to the urine sample itself, influenced the rats' behaviour. For example, there may have been effects of separation from familiar cage-mates and/or of social isolation (in the continued, unavoidable presence of the odour of an unfamiliar conspecific), and these could have acted as non-specific disruptive stimuli, as appeared to be the case with the 'handling' treatment (see below).

The effect of handling (a non-specific stimulus) on the memory trace is more likely to reflect an indirect stress effect on memory. Handling appears to be a stressful procedure for rodents (eg Rodent Refinement Working Party 1998). Increases both in dopamine activity in the medial prefrontal cortex (associated predominately with stress responses) (eg Feenstra & Botterblom 1996), and in Fos (an immediate early gene) expression in areas directly mediating the stress response (eg Ryabinin *et al* 1999), are seen after handling. This is in addition to raised body temperature and reductions in food intake and body weight (eg Michel & Cabanac 1999). It also appears that handling during early life can have a profound and long-lasting influence on a rat's behavioural and physiological stress responses in later life (Anisman *et al* 1998).

The effect of handling on social memory may therefore be a consequence of the stress response initiated by the handling procedure. Such physiological stress responses have been demonstrated to have considerable impact on cognitive function (eg de Quervain *et al* 1998), including memory and attention (eg Mendl 1999). It is possible that recognition of even very familiar individuals could be disrupted, at least for the duration of time (15 min) tested in Experiment 2, as a result of the stress response elicited by handling. In our study, increasing experience of the handling treatment

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appeared to reduce its disruptive capability. This observation reflects that of Ryabinin *et al* (1999), in which repeated handling and sham injections resulted in complete habituation of the immunoreactive stress response in some strains of mice. However, in the present study, when the 'handling' treatment was extended to a duration not previously experienced by the subject rats (15 mins), this once again appeared to impair recognition.

The results of this study suggest that there is an apparent impairment of social memory following exposure to elements of husbandry procedures. However, we cannot conclude whether this active disruption of memory performance is permanent or temporary, or, if temporary, for how long such an impairment may continue — although it does appear to last over 15 mins. Permanent disruption of social memory, although unlikely after long periods of group housing, may lead to aggression between conspecifics as a consequence of the 'disturbed' rat having to reassert itself within the social group; the individuals of which it would no longer recognise. However, even if the impairment of memory performance is only brief, once an animal has been returned to its social group it may (for however long) be unable to discriminate between the odours of its groupmates. Throughout this period it might therefore be vulnerable to increased aggression and agonistic interaction because of its inappropriate behaviour when interacting with group members, and this could result in reduced welfare. We can therefore speculate that during the initial period after reintroduction to a stable group, rats that have experienced a disruptive husbandry procedure should show a change in social behaviour. This is an area of research that we are currently investigating.

A potential problem with using a habituation/discrimination test of this kind is that there can be difficulty with the interpretation of negative results. Whilst a statistically significant preference to investigate the novel stimulus can be interpreted relatively reliably as a recognition of, and habituation to, the original stimulus, any failure to show a discrimination is open to a number of explanations other than that the original stimulus was not recognised. For example, a subject may reinvestigate the original stimulus in preference to the novel odour, even though it recognises the original stimulus, purely because the original stimulus has not been recently encountered following a period of separation. A failure to discriminate may also be explained by simple subject performance error (eg Wilkie *et al* 1999). As a consequence, the underlying assumption of the social discrimination procedure — that a subject will prefer to investigate a novel stimulus rather than a simultaneously introduced original (remembered) stimulus — may be confounded. As such, any negative results should be interpreted with caution. For example, a negative result should be described as "a failure to demonstrate discrimination" rather than as "a failure to discriminate" *per se*.

This problem is demonstrated in the present study by the lack of a significant statistical preference for the novel odour in the 'control' treatment after 7 days and two weeks of group housing. This means that we can never entirely rule out the possibility of alternative explanations for the observed effects other than that the implementation of treatments had impaired recognition processes. One such alternative explanation is that handling may have increased the motivation of subject rats to reassess a familiar (remembered) stimulus in preference to a novel stimulus. This might have occurred if the underlying assumed preference of a test rat for the novel stimulus was changed to a preference for the familiar (remembered) stimulus — perhaps because the stress of handling resulted in an increased need for the reassurance of a familiar companion (cf Kristensen *et al* 2001).

Other potential influences on the discrimination test include possible difficulties in discriminating between inbred individuals (eg Nevison *et al* 2000) and/or the creation of dominance relationships between sexually mature conspecifics (eg Carr *et al* 1976; Brown 1992) — although this is more likely to apply following extended periods of group housing. For example, Brown (1992) found that dominant adult male rats preferred the odour of familiar subordinate males to that of simultaneously introduced novel males, which contradicts the main assumption of the social discrimination procedure. This could explain why, in the current study, discriminations were less evident after 2 weeks of group housing, because by then the rats would have had sufficient time for the formation of a dominance hierarchy (eg 7 days [Hurst *et al* 1996]).

Despite these potential influences, the current study provides evidence that elements of standard husbandry practice can prevent, at least temporarily, the demonstration of discrimination between a familiar and an unfamiliar conspecific, even, in the case of handling, when rats have been group-housed for a week or more. The pattern of the results is as one might predict if disturbances were having some effect on memory performance that was proportional to: (1) the disturbance duration, and/or (2) the establishment of the memory. This may, at least in part, underlie the increases in aggression observed in previously stable social groups following routine husbandry procedures such as cage cleaning (eg Gray & Hurst 1995; Van Loo *et al* 2000). However, it is likely that other factors, such as novel odour deposition on handled individuals (either human or latex odours), may also play a role in the change in behaviour observed following procedures such as handling, and these require further investigation.

Animal welfare implications

The results of this study have several implications for the welfare of laboratory-housed rats, for other rodents and perhaps also for other species. (1) It appears that when rats are first grouped, they should be undisturbed for at least 24 h to prevent possible recognition failure as a result of exposure to handling or to the odours of novel conspecifics. (2) Handling may cause temporary or long-term interference with social memory in individuals unaccustomed to handling, even after animals have been housed together for more than 24 h. (3) As a consequence, when possible, rats should be gradually familiarised with handling, such that the disruptive/stressful effects of handling are reduced. (4)

After husbandry procedures, the behaviour of rats reintroduced to their social groups should be observed in order to ensure that any expression of inappropriate behaviour does not result in agonistic and potentially injurious behaviour.

If such steps are taken, in addition to potentially benefiting the welfare of laboratory rats by avoiding impairment of social discrimination processes, the value of experimental data obtained from the animals may also be improved, since data acquired from stressed animals and/or those with poor welfare may be compromised (eg Würbel 2001).

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References

Anisman H, Zaharia M D, Meaney M J and Merali Z 1998 Do early-life events permanently alter behavioral and hormonal responses to stressors? *International Journal of Developmental Neuroscience 16(3/4)*: 149-164

Beynen A C 1992 Communication between rats of experiment-induced stress and its impact on experimental results. *Animal Welfare 1*: 153-159

Brown R E 1992 Responses of dominant and subordinate male rats to the odors of male and female conspecifics. *Aggressive Behavior 18*: 129-138

Burman O H P and Mendl M 1999 The effects of environmental context on laboratory rat social recognition. *Animal Behaviour 58*: 629-634

Burman O H P and Mendl M 2000 Short-term social memory in the laboratory rat: its susceptibility to disturbance. *Applied Animal Behaviour Science 67*: 241-254

Carr W F, Yee L, Gable D and Marasco E 1976 Olfactory recognition of conspecifics by domestic Norway rats. *Journal of Comparative and Physiological Psychology 90*: 821-828

Dantzer R, Bluthé R M, Koob G F and Le Moal M 1987 Modulation of social memory in male rats by neurohypophyseal peptides. *Psychopharmacology 91*: 363-368

de Quervain D J F, Roozendaal B and McGaugh J L 1998 Stress and glucocorticoids impair retrieval of long-term spatial memory. *Nature 394*: 787-790

Dluzen D E, Muraoka S, Engelmann M, Ebner K and Landgraf R 2000 Oxytocin induces preservation of social recognition in male rats by activating alpha-adrenoceptors of the olfactory bulb. *European Journal of Neuroscience 12*: 760-766

Dluzen D E, Muraoka S, Engelmann M and Landgraf R 1998 The effects of infusion of arginine vasopressin, oxytocin, or their antagonists into the olfactory bulb upon social recognition responses in male rats. *Peptides 19*: 999-1005

Dukas R 1998 Constraints on information processing and their effects on behaviour. In: Dukas R (ed) *Cognitive Ethology — The Evolutionary Ecology of Information Processing and Decision Making* pp 89-127. University of Chicago Press: Chicago, USA

Engelmann M, Wotjak C T and Landgraf R 1995 Social discrimination procedure — an alternative method to investigate juvenile recognition abilities in rats. *Physiology and Behavior 58*: 315-321

Ewbank R and Meese G B 1971 Aggressive behaviour in groups of domesticated pigs on removal and return of individuals. *Animal Production 13*: 685-693

Feenstra M G P and Botterblom M H A 1996 Rapid sampling of extracellular dopamine in the rat prefrontal cortex during food consumption, handling and exposure to novelty. *Brain Research 742*: 17-24

Fokkema D S, Koolhaas J M and van der Gugten J 1995 Individual characteristics of behavior, blood pressure, and adrenal hormones in colony rats. *Physiology and Behavior 57(5)*: 857-862

Gray S and Hurst J L 1995 The effects of cage cleaning on aggression within groups of male laboratory mice. *Animal Behaviour 49*: 821-826

Hurst J L, Barnard C J, Hare R, Wheeldon E B and West C D 1996 Housing and welfare in laboratory rats: time-budgeting and pathophysiology in single-sex groups. *Animal Behaviour 52*: 335-360

Kristensen H H, Jones R B, Schofield C P, White R P and Wathes C M 2001 The use of olfactory and other cues for social recognition by juvenile pigs. *Applied Animal Behaviour Science 72*: 321-333

Lapin I P 1995 Only controls — effect of handling, sham injection, and intraperitoneal injection of saline on behavior of mice in an elevated plus-maze. *Journal of Pharmacological and Toxicological Methods 34*: 73-77

Macphail E M 1986 Animal memory: past, present and future. *The Quarterly Journal of Experimental Psychology 38(B)*: 349-364

Mendl M 1999 Performing under pressure: stress and cognitive function. *Applied Animal Behaviour Science 65*: 221-244

Michel C and Cabanac M 1999 Opposite effects of gentle handling on body temperature and body weight in rats. *Physiology and Behavior 67*: 617-622

Minitab 1996 *Minitab Reference Manual, Version 12*. State College Philadelphia: Philadelphia, USA

Nevison C M, Barnard C J, Beynon R J and Hurst J L 2000 The consequences of inbreeding for recognizing competitors. *Proceedings of the Royal Society of London, Series B, Biological Sciences 267*: 687-694

Noldus Information Technology 1993. *The Observer Base Package. Reference Manual, Version 3.0*. Noldus Information Technology: Wageningen, The Netherlands

Popik P and van Ree J M 1998 Neurohypophyseal peptides and social recognition in rats. *Progress in Brain Research 119*: 415-436

Reijmers L G J E, Leus I E, Burback P H, Spruijt B M and van Ree J M 2001 Social memory in the rat: circadian variation and effect of circadian rhythm disruption. *Physiology and Behavior 72*: 305-309

Rodent Refinement Working Party 1998 Refining rodent husbandry: the mouse. *Laboratory Animals 32*: 233-259

Rodriguez W A, Borbely L S and Garcia R S 1993 Attenuation by contextual cues of retroactive interference of a conditional discrimination in rats. *Animal Learning and Behavior 21*: 101-105

Ryabinin A E, Wang Y M and Finn D A 1999 Different levels of Fos immunoreactivity after repeated handling and injection stress in two inbred strains of mice. *Pharmacology Biochemistry and Behavior 63(1)*: 143-151

Sales G D 1991 The effect of 22 kHz calls and artificial 38 kHz signals on activity in rats. *Behavioural Processes 24*: 83-93

Sawyer T F, Hengehold A K and Perez W A 1984 Chemosensory and hormonal mediation of social memory in male rats. *Behavioural Neurosciences 98*: 908-913

Schmitt U and Hiemke C 1998 Combination of open field and elevated plus-maze: a suitable test battery to assess strain as well as treatment differences in rat behavior. *Progress in Neuro-Psychopharmacological and Biological Psychiatry 22*: 1197-1215

Sokal R R and Rohlf F J 1995 *Biometry*. W H Freeman and Company: New York, USA

Squire L R 1986 Mechanisms of memory. *Science 232(4758)*: 1612-1619

Squire L R 1987 *Memory and Brain*. Oxford University Press: Oxford, UK

Van Loo P L P, Kruitwagen C L J J, Van Zutphen L F M, Koolhaas J M and Baumans V 2000 Modulation of aggression in male mice: influence of cage cleaning regime and scent marks. *Animal Welfare 9*: 281-295

Wilkie D M, Willson R J and Carr J A R 1999 Errors made by animals in memory paradigms are not always due to failure of memory. *Neuroscience and Biobehavioral Reviews 23*: 451-455

Würbel H 2001 Ideal homes? Housing effects on rodent brain and behaviour. *Trends in Neurosciences 24(4)*: 207-211