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Corticosterone differences rather than social housing predict performance of T-maze alternation in male CD-1 mice

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Abstract

This study examined the effects of social housing manipulations on bodyweight, corticosterone levels, and performance of T-maze alternation in male CD-1 mice. Males that adopted a dominant social rank were heavier than those that adopted a subordinate social rank. Dominant males also had lower corticosterone concentrations than the subordinates. However, there was little to suggest that these physiological indicators of social rank were moderated by housing condition. Indeed, statistical analysis confirmed that the difference in bodyweights was evident before males were socially housed. The mice showed high levels of spatial alternation on the T-maze from the start of testing so performance accuracy was high. Neither social rank nor housing condition had any clear categorical effect on T-maze performance. However, performance did fluctuate over successive blocks of testing and there was a negative association between accuracy on the T-maze and corticosterone levels (consistent with performance impairment because of elevated corticosterone). Therefore, under present conditions, individual differences in corticosterone were a better predictor of T-maze performance than social rank or housing condition. The results of the present study lend further support to the proposition that corticosterone levels measured non-invasively in urine may be used to predict diverse welfare outcomes for laboratory mice, from bodyweight to cognitive performance. Moreover, intrinsic physiological parameters rather than external influences, such as social housing, may have more influence on mouse behaviour.

Keywords: *animal welfare, CD-1 mouse, social housing, social rank, T-maze alternation, urinary corticosterone*

Introduction

Despite the increased risk of aggressive encounters, a number of studies advocate the use of group housing for rodents (Valzelli *et al* 1977; Ikemoto & Panksepp 1992; Gray & Hurst 1995; Hurst *et al* 1997; Jennings *et al* 1998; van Loo *et al* 2000, 2004; Suckow *et al* 2001). One reason for this recommendation is that isolated mice have been shown to display a number of deleterious behavioural and physiological alterations (Koyama 1993, 1995; Haseman *et al* 1994; Wu *et al* 2000; Bartolomucci *et al* 2003a; Guo *et al* 2004) compared to group-housed subjects. These alterations have been termed the 'isolation syndrome' (Valzelli 1973). Thus, the effects of group housing on behavioural and physiological parameters are likely to impact on a range of welfare parameters.

Earlier studies have compared learning ability in socially- and singly-housed rodents, but findings have, to date, been mixed. Some have found evidence for cognitive impairment in isolated rodents (Valzelli *et al* 1977; Lu *et al* 2003; Elliott & Grunberg 2005; Sandstrom & Hart 2005; Chida *et al* 2006); others have demonstrated that, under some circumstances, isolated individuals perform better than those that are socially housed (Wongwitdecha & Marsden 1996; Moragrega *et al* 2003, 2005; Hermes *et al* 2005); and, depending on the learning measure in use, there can be no difference between isolated and group-housed mice (Coudereau *et al* 1997; Krohn *et al* 2006).

Social rank differences — that are more pronounced in mice than rats — may go some way towards explaining these discrepancies. When male mice are housed together they generally engage in aggressive interactions to establish a dominance hierarchy (Crowcroft 1966; Poole & Morgan 1973, 1976; Mondragón *et al* 1987; Collins *et al* 1997). Social rank differences are associated with a number of behavioural and physiological differences (eg Desjardins *et al* 1973; Kudryavtseva *et al* 1991; Martínez *et al* 1998; Lumley *et al* 1999; Bartolomucci *et al* 2001, 2003b, c, 2004, 2005). Performance in learning tasks may also be affected (Barnard & Luo 2002; Spritzer *et al* 2004; Fitchett *et al* 2005a, 2006). Furthermore, studies have also found

that the negative consequences of living in a stressful social environment may persist in subordinate males for days, even weeks, after interactions have stopped (Koolhaas *et al* 1990, 1997; Tornatzky & Miczek 1993; Meerlo *et al* 1996a, b, c; Ruis *et al* 1999; Lucas *et al* 2004; Buwalda *et al* 2005; De Jong *et al* 2005; Fitchett *et al* 2005a; Berton *et al* 2006). The majority of these studies used social defeat protocols where males are exposed to brief periods of attack from a larger, more aggressive male, and are then removed to a separate home cage away from the aggressive male. However, the interactions that arise in the course of normal social interactions of group housing can also have long-lasting effects (Fitchett *et al* 2005a).

We found that subordinate mice that had been separated from their cage mate, due to excessive aggressive interactions, showed persistent deficits on a T-maze task that were not remedied by re-housing subordinates as singletons away from their dominant cage mate (Fitchett *et al* 2005a). Only particularly aggressive pairings were separated in this earlier study. Therefore, in the present study, we tested the effects of social rank and housing conditions on performance in the same T-maze alternation task under conditions in which aggression levels were lower and matched pairs of mice could be selected to test under different housing conditions. In the previous studies, elevated urinary corticosterone predicted later subordination, consistent with intrinsic difference in the stress responsiveness of the mice which turned out to be particularly subject to social defeat (Fitchett *et al* 2005a, b). Therefore, the present study also examined social rank and performance on T-maze alternation in relation to differences in urinary corticosterone.

Materials and methods

Animals

Subjects were 60 male CD-1 mice (Harlan Ltd, Oxon, UK), aged six weeks at the time of delivery. Mice were marked with black eyelash dye (Colorsport 30 Day Mascara, Brodie and Stone Plc, UK) to enable individual identification. Two animals were excluded because they did not run on the T-maze (1 separated subordinate and 1 isolated mouse).

Housing conditions

On arrival, all mice were singly housed in standard, opaque, polypropylene laboratory cages (48 × 15 × 13 cm [length × breadth × height]; model M3, North Kent Plastics, UK) for two weeks. A 12:12 reversed light/dark cycle (white lights on 2030–0830h) allowed all behavioural observations to be done during normal working hours in the dark (active) phase under dim (40 W) red lighting. Mice were fed standard laboratory mouse diet (Harlan Ltd, Oxon, UK), *ad libitum* with the exception that food was removed 3 h prior to T-maze testing, to motivate responding. Tap water was available *ad libitum* in the home cage. Cages contained sawdust and environmental enrichment was provided in the form of shredded tissue as nesting material and cardboard tubes.

This settling period as singletons was necessary to allow a suitable baseline determination of corticosterone levels before any social hierarchy developed. At week three, 10 males were assigned to the isolated housing condition using the random number generator in Microsoft Excel®: these individuals remained singly housed throughout the experiment in standard cages as above. Attempts were made to pair-house the remaining 50 individuals, as above, in standard cages following a previously established procedure (Fitchett *et al* 2005b).

In total, 17 dyads were created: 14 dyads at the first attempt and three at the second attempt. Initial allocation to a dyad was random, at the second attempt selection for pairings was based on a semi-random allocation (from amongst the mice which needed to be re-paired). At week five, eight of the dyads that had been created were separated and re-housed as singletons in the same standard cages. The dyads to be separated were selected on the basis of behavioural data collected over the preceding two-week social rank establishment period, so that there were no differences in initial aggression levels between dyads that were separated and those that remained paired (behavioural ratings reported below). The remaining nine dyads were pair-housed for the rest of the experiment. In summary, by week five, three housing conditions had been established: 'socially isolated' (n = 10), 'pair-housed' (n = 18) and 'separated' (n = 16). Figure 1 shows a timeline of the methods used in this experiment.

Rank-related behaviours and housing

During weeks three and four, when both the paired and separated groups were socially housed, daily observation sessions (30 min) recorded the number of aggressive and submissive behaviours to determine the dominant and subordinate male in each dyad (Fitchett *et al* 2005a). The aggressive and submissive behaviours were adapted from (Mackintosh 1981) and are summarised in Table 1.

At the end of this two-week period, these data were used to ensure that mice assigned to the paired and separated conditions were matched in terms of initial aggression levels.

Urine collection

Two urine samples were collected. Urine sample A was collected over six days during week two, when the mice were singly housed. Urine sample B was collected over six days during week seven. Thus, both urine samples were cumulative. Urine collections were carried out during the middle part of the day in a testing room separate from the holding room. Mice were moved at the beginning of each collection day and returned to the holding room at the end of the day. Each mouse was placed individually into an empty, opaque, polypropylene cage (33 × 15 × 13 cm [length × breadth × height], North Kent Plastics, UK) for 30 min per day of the collection period (six days) and all urine produced was collected using a 1 ml syringe and needle (Becton Dickinson UK Ltd, UK), and stored at –20°C until analysis. Urine from each day of the collection period was pooled for each individual, until a suitable sample volume was reached, in most cases 0.5 ml, although if this was not possible smaller samples were assayed. To control for the amount of urine produced, creatinine was also assayed (Dahlborn 1996; Brennan *et al* 2000; Muir *et al* 2001; van Loo *et al* 2001a, 2002, 2003; Touma *et al* 2003).

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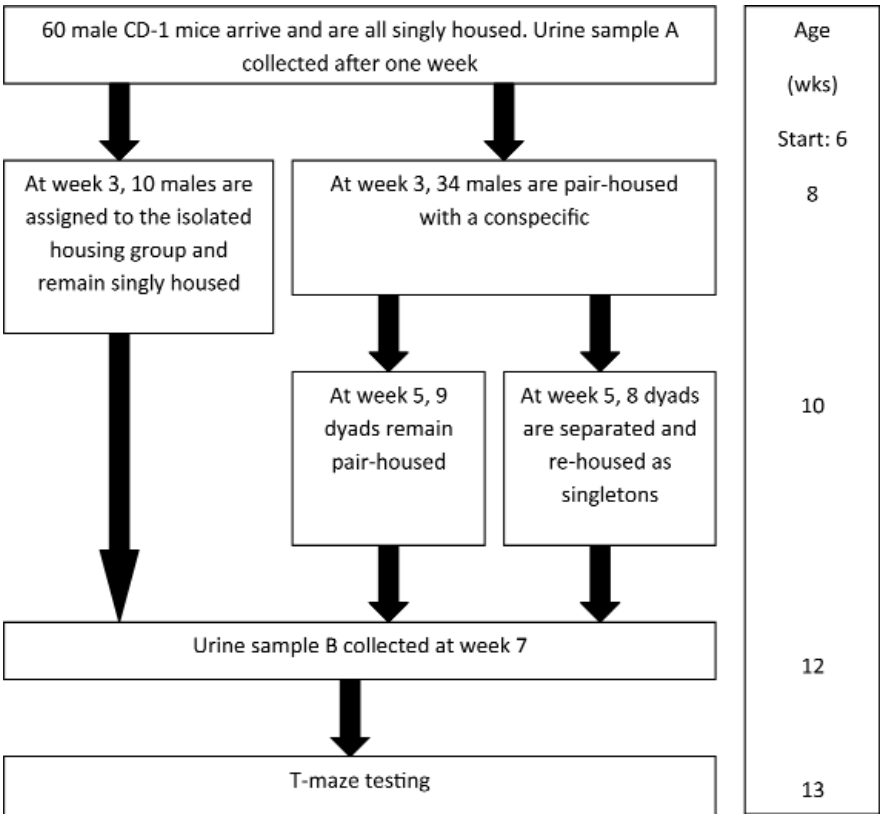
Figure 1



The timeline of the study. Right-hand panel shows cumulative time elapsed in weeks.

In the period between urine samples A and B, the paired group had been housed in dyads for four weeks, the separated group had been housed in a dyad for two weeks and re-housed as singletons for two weeks; the isolated group were singly housed throughout the experiment (Figure 1). All samples were assayed for corticosterone and creatinine levels (Fitchett *et al* 2005a, b). Four corticosterone samples were excluded because the urine volume collected post-pairing was too low for assay.

Urinary corticosterone was measured using an adapted commercial enzyme immunoassay kit (Correlate-EIA, Assay Designs, MI, USA). Samples were assayed after dilution 1/50 with assay buffer using a ROSYS PLATO (Robotec, UK) system automatically performing all pipetting, incubation and measurement stages for the assays. Urinary creatinine was analysed by an automated, modified Jaffe reaction, using a COBAS MIRA clinical analyser (ABX, UK). Quality control samples were run with each batch of urine samples for creatinine and at the beginning and end of each immunoassay microtitre plate for corticosterone assays. Urine corticosterone results were reported corrected for creatinine content to control for differences in urine production rate and hydration status. Corticosterone values are therefore reported as mg mol^{-1} creatinine.

Table 1 Scoring system used for aggressive and submissive behaviours during weeks 3 and 4 in which there were regular 30 min observation sessions of dyads (based on Mackintosh 1981).

Aggressive behaviours	Submissive behaviours
Threat	Evade
Aggressive groom	Retreat
Bite	Flee
Over	On back
Chase	Oblique posture
Rattle	Kick
Circle	Crouch
Zig-zag	Straight legs
Walk round	On bars
	Off bars freeze

T-maze tests

The T-maze was made of wood and consisted of a central stem measuring 80 × 10 cm (length × breadth) and a left and right arm both measuring 60 × 10 cm. This platform was at a height of 30 cm from the ground. At the end of

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each of the choice arms was a food well, into which sunflower seeds were placed. Mice were given one habituation session which consisted of five min free exploration with both choice arms baited with sunflower seeds. Testing began 24 h later and mice received two trials per day for 15 days. Each trial consisted of two parts: the first was a forced-choice run, in which only one arm of the T-maze was accessible; when the mouse entered this arm a reward was placed into the food well. This was followed by a free-choice run in which both arms were accessible although mice were only rewarded if they correctly alternated and entered the arm which had been blocked on the forced-choice run. If no choice was made after five min, mice were removed from the apparatus. The time taken to make a choice on forced- and free-choice runs was recorded as well as whether mice correctly alternated. The apparatus was wiped with diluted detergent between each run and rewards were not placed into the wells until after a choice had been made, to control for odour cues. The number of left and right trials was counterbalanced across testing.

Statistical analysis

All analyses were performed using SPSS (version 12.0.1; SPSS Inc, Illinois, USA) in a mixed design. The between groups factors were housing and social rank. It was necessary to conduct separate analyses to examine the effects of housing condition (at three levels: isolated, paired, and separated) because mice in the isolated group did not experience social interactions. However, analyses of the effects of social rank (at two levels: dominant or subordinate) included the relevant housing conditions (this factor, now at two levels: paired and separated) to test whether any effects of social rank were moderated by housing condition. The repeated measures factors were week (for successive determinations of weight); sample (for successive corticosterone assays) or six blocks of five trials testing on the T-maze (as per Fitchett *et al* 2005a), as applicable.

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 minimal (Howell 2002).

The relationship between overall performance accuracy and corticosterone measures was tested by correlational analysis (Pearson, two-tailed).

The results for the three phases of the study are presented in turn. The first is the pre-pairing data from weeks 1–2: bodyweights and results of assays on urine sample A. The second phase is the post-pairing data from weeks 3–7: bodyweights, behavioural observations and results from assays on urine sample B. The third phase is the T-maze data collected over weeks 8–10; the correlation with urinary corticosterone post-pairing and the change in urinary corticosterone from sample A to sample B; as well as a final analysis of bodyweight differences.

Results

Pre-pairing data (weeks 1–2)

Bodyweights

Figure 2 shows how bodyweight changed depending on (a) housing condition and (b) social rank over the duration of the experiment. Bodyweights collected before pairing were analysed with the repeated measures factor of week (at two levels as two weights were taken before pairing, 1 per week), and the between groups factor of later housing condition (at three levels: isolated, paired and separated). This showed no change in bodyweight during this period, with no difference by later housing condition (all *F*-values < 1). Therefore, mice were well matched in terms of bodyweight across the housing condition allocations (Figure 2[a], pre-pairing). A second repeated measures ANOVA with the factors of later social rank (dominant or subordinate) and later housing condition (at two levels: paired or separated) suggested that bodyweight was a predictor of later social rank. This showed an interaction between week and later social rank ($F_{1,30}$ = 5.704, P = 0.023). Mice which would later become dominant showed some increase, mice which would later become subordinate showed some decrease in weight between weeks 1 and 2 (Figure 2[b], pre-pairing). There was also a main effect of later social rank on pre-pairing bodyweight ($F_{1,30}$ = 9.219, P = 0.005): overall, mice which would later be dominant were heavier than mice which would later be subordinate. Again, there were no effects of later housing either on its own or in interaction (maximum $F_{1,30}$ = 2.780).

Urine assay for corticosterone

There was no overall effect of housing condition-to-be ($F_{2,41}$ = 0.060). Therefore, the mice were well matched across allocation to the different housing conditions. A second analysis that included later social rank as well as housing condition as factors, showed a marginal main effect of later social rank ($F_{1,30}$ = 3.53, P = 0.07) because the mice which would become subordinate tended to have overall higher urinary corticosterone levels. This suggestion of intrinsic difference was confirmed at the post-pairing assay (see below). There was no interaction between later social rank and housing condition-to-be ($F_{1,30}$ = 1.35).

Post-pairing data (weeks 3–7)

Ratings of rank-related behaviours

The number of aggressive and submissive behaviours scored during weeks three and four was used to identify the dominant and subordinate in each dyad, and also to identify which dyads to separate and which to leave paired (Table 2).

ANOVA confirmed that dominant and subordinate mice were clearly identifiable. As would be expected, there was a clear effect of social rank on both the number of aggressive ($F_{1,32}$ = 17.82, P < 0.001) and submissive behaviours ($F_{1,32}$ = 18.60, P < 0.001) scored during weeks three and four, during which dominance was established within the dyads.

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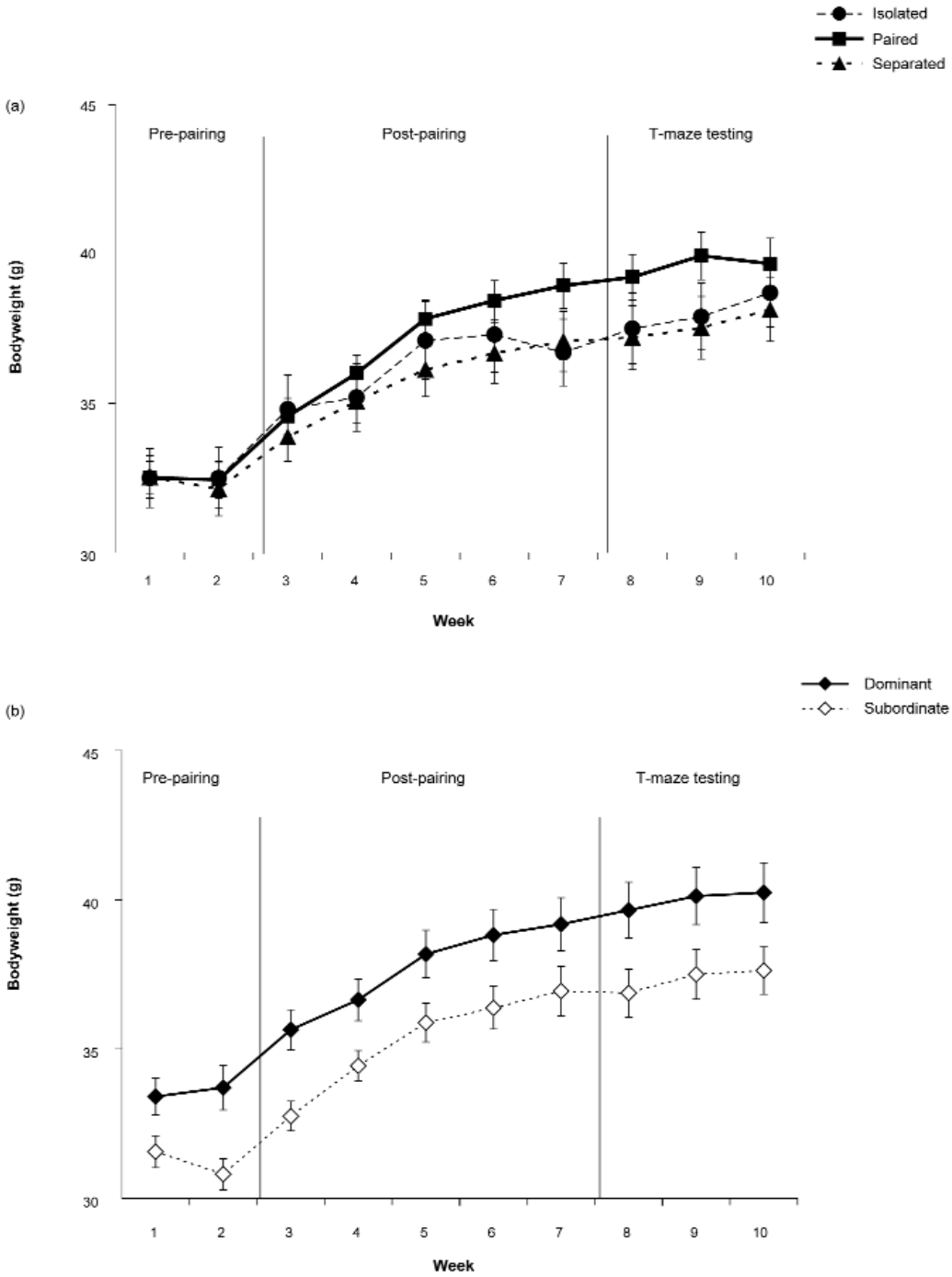
Figure 2



Bodyweight (g) changes during the different stages of the study by (a) housing condition and (b) social rank.

Confirming that the allocation to paired and separated housing groups was well-matched, there was no difference in the number of aggressive or submissive behaviours by housing condition, both F-values < 1.

Bodyweights

Bodyweights were again analysed with the repeated measures factor of week (at five levels as five weights were taken, one per week), first with the between groups factor of housing condition. There was a significant interaction

Table 2 Mean (± SE) aggressive and submissive behaviours recorded during behavioural observation sessions by social rank and housing condition.

Group	Aggressive behaviours	Submissive behaviours
Dominants (n = 17)	15.1 (± 3.19)	0.9 (± 0.59)
Subordinates (n = 17)	1.4 (± 0.66)	14.8 (± 3.15)
Paired (n = 18)	8.0 (± 2.42)	7.7 (± 2.36)
Separated (n = 16)	8.5 (± 3.36)	8.1 (± 3.37)

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Table 3 Mean (± SE) concentrations of corticosterone (mg mol^{-1}) as determined from the pre- and post-pairing urine samples, shown separately by social rank and housing condition.

Group	Pre-pairing	Post-pairing
Dominant (n = 17)	7.3 (± 0.97)	3.1 (± 0.36)
Subordinates (n = 15)	13.3 (± 2.97)	4.9 (± 1.20)
Isolated (n = 8)	10.0 (± 1.29)	2.4 (± 0.54)
Paired (n = 17)	10.1 (± 1.08)	3.6 (± 0.37)
Separated (n = 15)	10.1 (± 3.14)	4.4 (± 1.23)

between week and housing condition ($F_{8,164}$ = 3.201, P = 0.002). Figure 2[a] shows that this interaction arose because the paired group gained more weight. Despite this effect of social housing on the rate of weight gain, there was no overall effect of housing condition ($F_{2,41}$ = 0.874).

As above, a second repeated measures ANOVA used social rank and housing condition as factors. Bodyweight of all mice significantly increased during this period, resulting in a main effect of week ($F_{4,120}$ = 77.378, P < 0.001). This weight gain did not vary according to social rank ($F_{4,120}$ = 2.201). There was, however, an overall effect of social rank ($F_{1,30}$ = 6.644, P = 0.015): as was the case pre-pairing, dominants were heavier than subordinates (Figure 2[b]). As above, there was no overall effect of housing ($F_{1,30}$ = 2.195) and no interaction between social rank and housing condition ($F_{1,30}$ = 0.409).

Urine assay for corticosterone

As might be expected, pre- and post-pairing corticosterone concentrations were significantly correlated (r_{40} = 0.732, P < 0.001). Corticosterone concentrations from urine sample A (pre-pairing) and B (post-pairing) were also compared using a repeated measures ANOVA with housing condition as the factor. This showed an effect of sample in that there was a significant change in corticosterone concentrations between the two assays ($F_{1,37}$ = 41.235, P < 0.001). Table 3 shows that urinary corticosterone concentrations were much lower at the second assay. There was no effect of housing, either overall or in interaction with sample (both F-values < 1).

The second repeated measures ANOVA looked at the effect of sample on corticosterone concentrations with both social rank and housing condition as factors. The effect of sample was still significant, as above ($F_{1,28}$ = 30.165, P < 0.001). Importantly, however, the evidence for intrinsic difference was confirmed by the overall effect of social rank ($F_{1,28}$ = 4.090, P = 0.05). Table 3 shows that overall subordinates had higher corticosterone concentrations than dominants. Although the drop in corticosterone post-pairing was bigger in subordinates, the interaction between sample and social rank did not reach significance ($F_{1,28}$ = 3.533, P = 0.07) and there were no significant effects or interactions involving housing condition (maximum $F_{1,28}$ = 1.473).

T-maze performance (weeks 8–10)

Bodyweights

Bodyweights collected during T-maze testing were analysed with the repeated measures factor of week (at three levels as three weights were taken, one per week) in a repeated measures ANOVA to first test for differences by housing condition. There was a main effect of week ($F_{2,80}$ = 13.551, P < 0.001). Overall, mice continued to increase in weight during the T-maze testing (Figure 2[a]). There was no effect of housing either overall or in interaction with week (maximum $F_{4,80}$ = 2.310).

Again, a second repeated measures ANOVA was conducted to test for effects of social rank. As reported for the full sample above, there was an effect of week ($F_{2,58}$ = 6.639, P = 0.002) but not in interaction with social rank or housing condition (maximum $F_{2,58}$ = 2.561). There was, however, an overall effect of social rank ($F_{1,29}$ = 5.143, P = 0.031) as dominants remained heavier than subordinates (Figure 2[b]). As above, there was no effect of housing condition (maximum $F_{1,29}$ = 2.916, ns).

T-maze performance

The 30 T-maze test sessions were analysed in six blocks of five trials in a repeated measures ANOVA with blocks at six levels, first with housing condition between subjects. The dependent variable was choice accuracy. Figure 3 shows that performance started high with the mice scoring the maximum possible number correct at the start of training. This reflects a high level of spontaneous alternation as the mice had no pre-training, just a single habituation session on the apparatus. However, performance was not at ceiling in that there was later fluctuation in performance over successive testing blocks, reflected in a main effect of blocks ($F_{5,195}$ = 8.320, P < 0.001), and a significant interaction between blocks and housing condition ($F_{10,195}$ = 2.200, P = 0.019). Differences emerged first at block four, where both paired and isolated groups outperformed the separated mice, minimum (t_{22} = 2.288, P = 0.032), but this difference was non-systematic in that performance of the separated mice subsequently recovered. There was no overall effect of housing condition ($F_{2,39}$ = 1.726).

The second repeated measures ANOVA included the social rank factor. There was a main effect of blocks ($F_{5,145}$ = 4.519, P = 0.001), as reported for the full sample above. There was no effect of social rank, either on its own or in interaction with blocks or housing (maximum $F_{5,145}$ = 1.832, ns). Again there was no overall effect of housing condition ($F_{1,29}$ = 3.152).

Thus, performance levels were high and what variability there was (reflected in the main effect of blocks) did not relate to housing or social rank. Nonetheless, the overall number of correct responses on T-maze tests was inversely correlated with post-pairing corticosterone concentration (r_{38} = –0.390, P = 0.015), so the greater the number of correct responses, the lower the corticosterone concentration. This was confirmed by analysis of a difference score to

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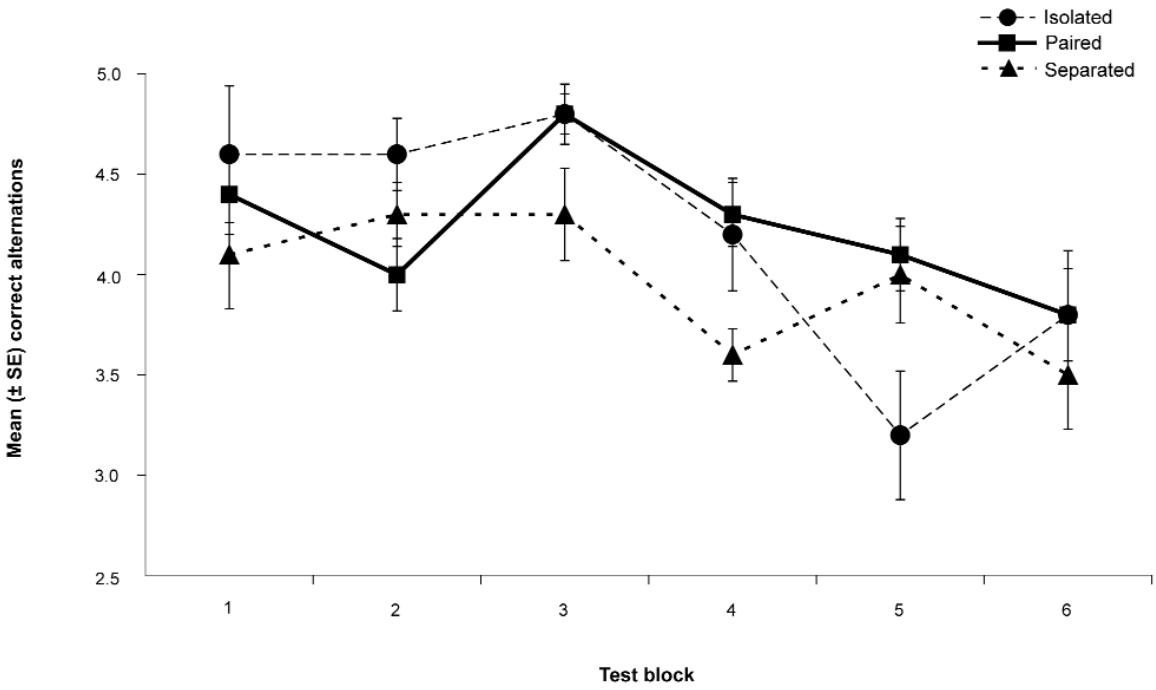
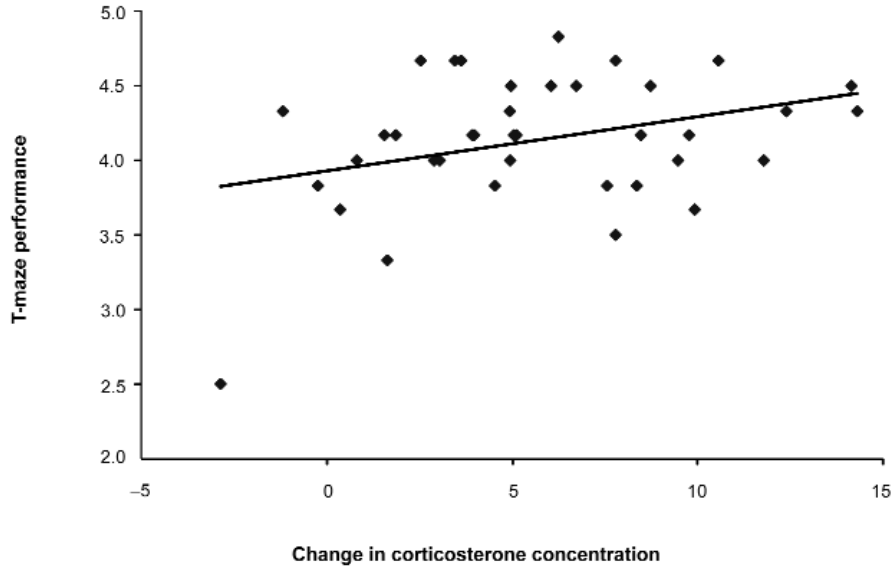
Figure 3



Mean (± SE) correct alternations at each test block by housing condition. A score of 5 reflects the maximum alternation score; a score of 2.5 reflects chance level performance.

Figure 4



The correlation between T-maze performance scored as the overall mean for correct alternations and the change in corticosterone concentration from the pre- to the post-pairing assay.

adjust for individual differences pre-pairing: the number of correct alternations was positively correlated with the change in corticosterone concentration from the pre- to the post-pairing assay ($r_{38} = 0.340$, $P = 0.037$), so the greater the number of correct alternations, the greater the drop in corticosterone concentration between the two assays (Figure 4).

Discussion

As expected, the mice clearly polarised into dominant and subordinate members of each dyad. Importantly, there were no effects of later housing condition on bodyweights or corticosterone concentrations during the pre-pairing period, so the mice were well matched across the different

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housing condition allocations. To ensure this matching, there were a number of differences between the present and the previous study (Fitchett *et al* 2005a) as a result of procedural changes (including the delay between housing manipulations and T-maze testing) intended to reduce the possibility that aggression levels would escalate. In the previous study, aggression levels became so high there was a risk of injury which made it necessary to separate some dyads on a non-random basis (Fitchett *et al* 2005a). Thus, in the present study, aggression levels were comparatively lower because separation was not necessary.

In the earlier study, the subordinates showing impaired accuracy in spatial alternation in the same T-maze task were from the more aggressive dyads and had particularly elevated corticosterone levels (Fitchett *et al* 2005a). In the present study, although there were clear physiological differences by social rank (in terms of bodyweight and corticosterone levels) there were no differences in T-maze performance by social rank. In particular, there was no performance impairment on the T-maze in mice classified as subordinate. However, correlational analyses showed that accuracy was nonetheless improved in mice with relatively lower post-pairing corticosterone levels. Comparison with earlier findings in the same procedure (Fitchett *et al* 2005a) suggests that categorical effects by social rank may only be demonstrable when aggression levels are relatively high (Koolhaas *et al* 1990, 1997; Tornatzky & Miczek 1993; Meerlo *et al* 1996a, b, c; De Jong *et al* 2005). That differences in T-maze performance should depend on social contextual factors, such as the level of home cage aggression, is consistent with the view that investment in learning and performance should vary depending on the reproductive value of the learning outcome (Barnard & Luo 2002).

Physiological measures

Throughout the experiment, males adopting a dominant status were heavier than their subordinate conspecifics. However, although the weight advantage in dominants persisted during the period of pairing, social rank did not affect the rate of weight gain. Moreover, we found that the weight difference was evident even before mice were pair-housed. This evidence for intrinsic difference supports the hypothesis that relatively increased bodyweight may be a factor in determining the greater competitive ability of dominants compared with subordinates (van Zegeren 1980; Clutton-Brock *et al* 1988; Schüler & Renne 1988; Andersson 1994).

In contrast, there was little evidence that housing condition affected bodyweight at any point during the study. The single exception was that, during the phase in which housing condition was manipulated, the paired group gained more weight, consistent with welfare benefits of social housing (van Loo *et al* 2001b; Faraday 2002).

Urine assays showed that subordinates had overall higher corticosterone concentrations compared with dominants. This finding is typically related to the greater social stress experienced by low ranking animals (Louch & Higginbotham 1967; Wittenberger 1981; Blanchard *et al* 1993; Schulkin 1999; Avitsur *et al* 2001; Keeney *et al* 2001; Sloman *et al* 2002; Cacho *et al* 2003; Summers *et al* 2003). However, in line with our earlier findings (Fitchett *et al* 2005a, b), present results suggest that high corticosterone reflects an intrinsic difference, independent of social housing. Between the two urine assays, before and after housing conditions were manipulated, there was a large drop in corticosterone concentrations. This drop is most likely attributable to habituation to the laboratory environment; what matters is whether it was moderated by housing condition or social rank.

Although, as Table 3 shows, this drop was larger in subordinates, it did not significantly differ by housing condition or social rank. Analysis of urinary corticosterone across the two samples showed that subordinates had overall higher corticosterone concentrations than dominants. Thus, this pattern of effects is similar but not identical to what we found in the earlier study (Fitchett *et al* 2005a).

T-maze performance

There was some decline in accuracy (correct alternations) over successive blocks of trials. This decline in accuracy results most likely from a build-up of proactive interference from one trial to the next as the mice became confused with repeated arm visits (Cohen *et al* 1996; Bakanova *et al* 1997). There was some effect of housing condition but this seemed to be non-systematic variation in that performance in the separated group subsequently recovered.

Similarly, there were no overall effects of social rank on T-maze performance. However, across the different groups of mice, accuracy scores were inversely related to post-pairing urinary corticosterone levels: the greater the number of correct responses, the lower the corticosterone concentration. Using a difference measure to control for individual differences in baseline corticosterone levels, pre-pairing, confirmed this relationship: the greater the drop in corticosterone from pre- to post-pairing, the higher the accuracy scores.

In line with other studies, this would suggest that relatively higher corticosterone concentrations can have a negative effect on performance in learning tasks (McEwen & Sapolsky 1995; De Kloet *et al* 1999, 2002, 2005; Sapolsky 1999; McEwen 2004; Joëls *et al* 2004, 2006). Although no overall categorical effects of social rank were found on T-maze performance, corticosterone concentrations were higher in subordinate compared with dominant males. Thus, the lower urinary corticosterone — that predicts better T-maze accuracy — conforms to the dominant mice profile (see Table 3).

Animal welfare implications

The results of the present study lend further support to the proposition that non-invasive measures of corticosterone levels may be used to predict diverse welfare outcomes for laboratory mice, from bodyweight to cognitive performance (Lane 2006). Specifically, the present data provide additional confirmation of the viability of urinary corticosterone assays as an alternative to salivary and faecal assays. However, our data suggest that this averaged measure of stress responsiveness can be best determined prior to social housing and habituation to laboratory conditions. The

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majority of studies sampling corticosterone levels in relation to social housing have done so only some weeks after arrival in the laboratory and the results have been inconclusive (Krohn *et al* 2006). In the present study, more than one sample was needed to demonstrate the overall effect of social rank on corticosterone levels but, with time to adapt to conditions, we find that although the measure remains predictive, initially clear differences between animals are attenuated (see also Fitchett *et al* 2005a, b).

Consistent with our earlier study when aggression levels were low (Fitchett *et al* 2005a), group housing produced little in the way of possibly adverse effects in male CD-1 mice. However, neither was there any benefit of group housing. There was some effect of housing on weight gain in that this was relatively greater in the paired group (though with some differences by social rank, reported below), but no effect of housing on corticosterone levels.

Male mice classified as dominant and subordinate, on the basis of behavioural ratings, clearly differed in terms of bodyweight and corticosterone concentrations. Males that adopted a dominant social rank were heavier than those that adopted a subordinate social rank. Moreover, the link between social rank and bodyweight was evident before males were paired. Similarly, dominant males had overall lower corticosterone concentrations than subordinates. Consistent with earlier work, these findings suggest that there are intrinsic physiological differences between mice that will adopt different social ranks (Fitchett *et al* 2005a, b). Present findings confirm that intrinsic physiological parameters, rather than external influences such as social housing, can have more influence on mouse behaviour.

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