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Brief report

Subordinate male mice show long-lasting differences in spatial learning that persist when housed alone

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Abstract

In the wild, house mice live in social groups, whereas in the laboratory male mice are often singly housed. Environmental enrichment such as that provided by social housing has been argued to improve the cognitive performance of laboratory animals in experimental tests. The aim of the present study was to test the cost of aggressive social interactions on learning in male CD-1 mice. We found that subordinate mice from more aggressive dyads showed spatial learning impairment, measured as alternation on a T-maze. Learning impairments in subordinates have hitherto been presumed attributable to the animals' exposure to, and relative standing within, the social group. By contrast, the impairment we observed could not have been the result of recent social defeat because it persisted weeks later when the mice were housed alone. Elevated urinary corticosterone predicted later subordination, though paradoxically these abnormally high levels were reduced by pair housing.

Keywords: CD-1 mouse; Social rank; Spatial learning; Corticosterone

Social housing provides a natural enrichment that should promote normal behaviour (Latham & Mason, 2004; Sherwin, 1998). However, the benefits of social housing are different for animals of different social rank. In the laboratory, subordinate animals can show higher levels of stress hormones such as glucocorticoids, suggesting that they suffer greater adverse effects of social stress than dominants (Creel, Creel, & Monfort, 1996). Such effects have been examined in relation to dysregulation of the hypothalamic–pituitary–adrenal axis (HPA) and possible glucocorticoid resistance (Avitsur, Stark, & Sheridan, 2001; Gariépy, Rodriguiz, & Jones, 2002; Preil et al., 2001). Consistent with the view that the HPA axis can become dysfunctional, the effects of social defeat and subordination include reduced food and water intake and decreased mating behaviour (Martinez, Calvo-Torrent, & Pico-Alfonso, 1998).

With respect to cognitive abilities, social status is known to affect learning in a number of species (Drea & Wallen, 1999; Nicol & Pope, 1999), including mice (Barnard & Luo, 2002), though evidence in particular learning contexts can be mixed (Barnard & Luo, 2002; Spritzer, Meikle, & Solomon, 2004). The relationship between spatial learning and dominance is of particular interest because both are likely to predict reproductive success in locating mates and subsequently repelling competitors. There is evidence in mice of a modulating effect of status in at least one widely used spatial paradigm, the radial maze (Barnard & Luo, 2002). Such effects have yet to be examined in relation to corticosterone. Corticosterone can be measured non-invasively in urine (Dahlborn, van Gils, van de Weerd, van Dijk, & Baumans, 1996; Fitchett, Collins, Mason, Barnard, & Cassaday, in press; Touma, Sachser, Möstl, & Palme, 2003), providing an

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integrated picture of systemic levels with good predictive validity. For example, rodent urinary corticosterone levels are reliably elevated after exposure to stressors (e.g., inescapable shock Brennan, Ottenweller, Seifu, Zhu, & Servatius, 2000). Conversely, reductions in urinary corticosterone provide evidence that the use of nesting material as environmental enrichment can promote welfare in mice (Van Loo et al., 2003).

In the present study, we tested pairs of male mice for learning differences mediated by social status on a T-maze spatial alternation task (Bertholet & Crusio, 1991; Gerlai, 1998). Subjects were 30 6-week old male mice (26–30 g) of the outbred albino strain CD-1 (Harlan, UK). Mice were maintained under a reversed 12:12-h light/dark cycle (lights on 20.30–8.30 h). All testing was carried out during the dark, active phase. Initially mice were singly housed in NPK M3 cages (48 × 15 × 13 cm). Mice were marked with black eyelash dye (Colorsport 30 Day Mascara, Brodie and Stone Plc, London, UK) for individual identification. After 2 weeks' single housing, the mice were housed in pairs, in clean NBK M3 cages, to provide 15 dyads. The timeline of the study is shown in Fig. 1.

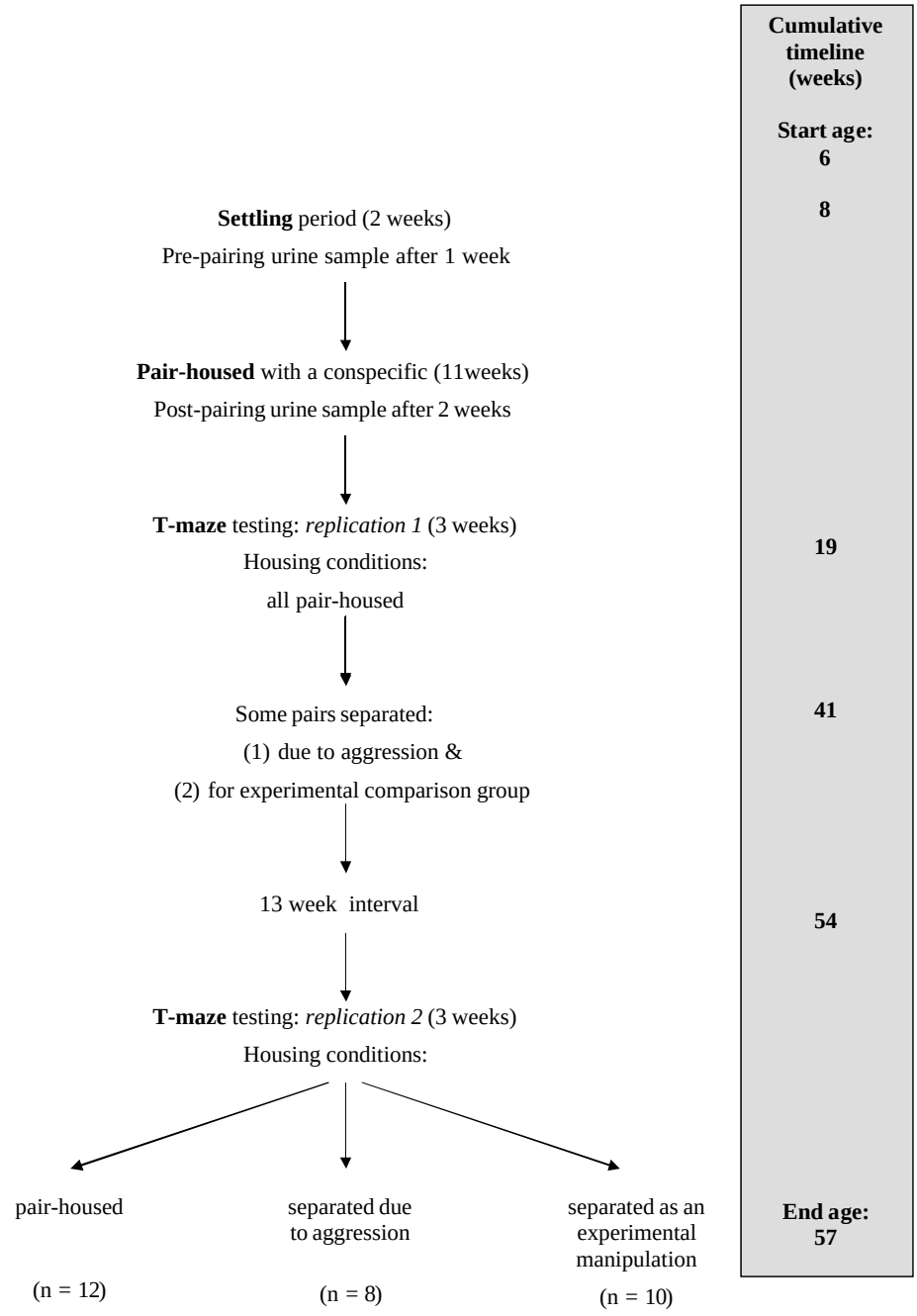

Fig. 1. The timeline of the study. The right-hand side panel gives the cumulative elapsed time in weeks.

Prior to behavioural testing, two urine samples were taken to assay for corticosterone levels. The first sample was taken after the first week of single housing and the second sample was taken after two weeks of pair housing. All urine collections were carried out during the middle portion of the dark active phase under dim red light. Mice were placed into individual clean cages ($33 \times 15 \times 13$ cm) for 30 min on each day of the collection period (6 days). Any urine produced was collected using a syringe and stored in a 1.5 ml eppendorf tube at −20 °C until analysis. Urine from each day was pooled for each individual until a suitable sample volume was reached, ideally 0.5 ml, although smaller samples were analysed. All assays were performed externally by the Health and Safety Laboratories, Sheffield, UK (for further details, see Fitchett et al., in press).

Behavioural tests of alternation on the T-maze began after 11 weeks of pair housing. The apparatus was a wooden T-shape with a central stem $80 \times 10 \times 1$ cm, and two choice arms both $60 \times 10 \times 1$ cm, at 30 cm above the ground. At the ends of both choice arms the height of the lip around the edge was 6.5 cm, food wells were 5 cm in from the ends of the two choice arms, 4 cm in diameter. A line was drawn on the stem to denote a start area, just large enough for the mouse to sit comfortably. Lines were also drawn two thirds of the way along both arms to provide the choice criterion.

After a single 5-minute habituation to the T-maze there followed 15 days' testing (conducted over 3 weeks), during which all the mice were pair housed. Each trial consisted of a forced run on which only one of the arms was accessible (and on which food was available), followed by a choice run on which both arms were accessible. Mice were only rewarded on the free choice run if they entered the arm that was different from that entered on the sample run (the choice criterion was that all four paws should cross the line two thirds along the arm). The apparatus was wiped with detergent between each run to remove urine and faeces. In the first replication, the delay between the sample and choice run was no more than was required to clean the maze (approximately 45 s). Mice received two trials per day, one to the left and one to the right side, in a semi-random sequence that was counterbalanced over days, so that half of all trials began with the left side blocked and half with the right side blocked.

We compared T-maze performance across two replications of testing on the T-maze conducted under different housing conditions, to test systematically how choice accuracy was influenced by the social environment. In the first replication, all mice were pair housed, but in the second replication 6 dyads were pair housed, 4 dyads had been separated due to aggression and 5 dyads had been separated as an experimental manipulation. This latter group allowed us to investigate the effects of separation without the confounding factor of aggression severity or time spent in pairs. There were a further 15 days' T-maze testing (again conducted over three weeks), procedures as above, except in the second replication, the time between the sample and choice run included an additional delay of 30 s, so there would be no ceiling effect in performance at this stage of learning. The interval between separating the mice and T-maze testing in replication 2 was at least 13 weeks.

A repeated measures ANOVA was used to test for differences in T-maze performance across the two replications of testing. Within-subjects factors were Replication (two levels) and Blocks of learning trials (six levels, each block containing five trials). Between subjects factors were Social status (subordinate or dominant) and Housing condition (pair-housed, aggressive separated, experimentally separated).

Learning was shown as an overall effect of Block ($F(5,120) = 12.55$, $p < .01$, across both replications of testing [mean correct alternations out of a possible 5] for replication 1: (mean ± SE) block $1 = 3.01 \pm 0.21$, block $6 = 4.05 \pm 0.18$; means for replication 2: block $1 = 3.33 \pm 0.25$, block $6 = 4.08 \pm 0.16$. There was a main effect of Housing condition, on T-maze alternation ($F(2,24) = 9.51$, $p = .001$) but this was dependent upon dominance status in that there was also a significant Social status × Housing interaction ($F(2,24) = 3.41$, $p = .05$). Fig. 2 shows that spatial alternation in dominants was not impaired in any housing condition, but subordinates in more aggressive pairings (that required separation) showed cognitive impairment. Social status was not significant as a main effect and did not interact with any other factor (maximum $F(5,120) = 1.43$). Moreover, as there was no effect of replication or interaction between replication and social status (both Fs < 1) this impairment was not improved by separation and the prevention of further aggressive encounters (see Fig. 3).

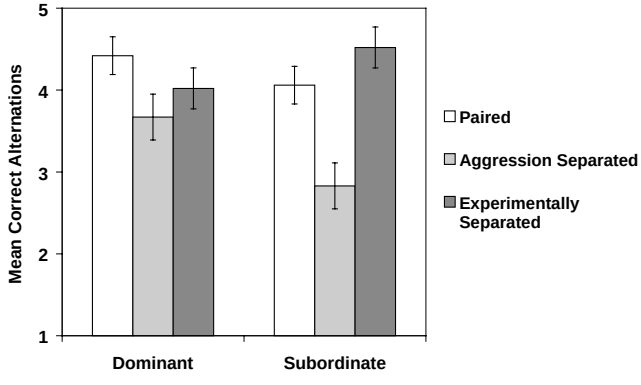

Fig. 2. Mean correct alternations out of possible 5, shown by social status and housing condition in replication 2. Error bars show the standard error of the mean. The interaction arose because although the dominant groups did not differ, maximum $t(9) = 1.58$, there were differences between subordinate mice as a function of housing condition. Whilst the performance of subordinate mice from the pair-housed and experimentally separated was equivalent, $t(6) = 1.91$, the aggressive separated subordinate mice performed worse than either of these, minimum $t(8) = 3.31$, $p = .01$.

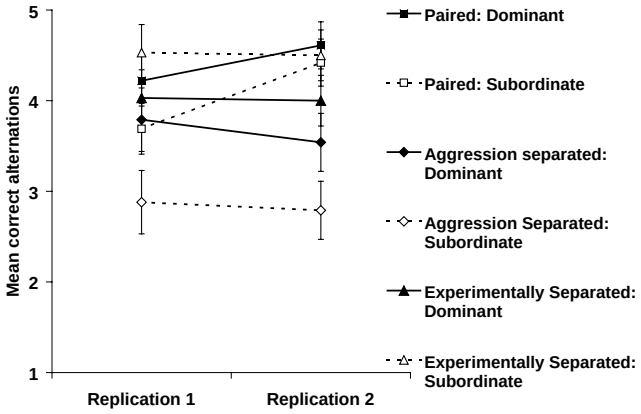

Fig. 3. Mean correct alternations (out of possible 5), shown by social status and housing condition, in replications 1 and 2. Error bars show the standard error of the mean. There were no significant effects of replication so the overall impairment seen in the aggressive separated subordinate mice was not reduced by single caging.

There was also very clear evidence that urinary corticosterone was affected by the social environment. This was analysed in a mixed design as a function of Pairing (pre- and post-), Social status (dominant and subordinate) and Housing condition-to-be (paired, aggressive separated and experimentally separated). This measure was taken before the T-maze tests, to investigate pre-existing differences between the later experimental housing groups.

Statistically, there was a significant interaction between Pairing, Social status and Housing condition, $F(2,22) = 3.76$, $p < .04$. Examination of the means shows that this interaction very obviously arises because of the large drop in corticosterone levels in the subordinates from the group that would later be separated because of high aggression, from pre-pairing 29.5 ± 4.26 to post-pairing 11.45 ± 2.14 (see Table 1). In all other groups, corticosterone levels were little altered by pairing.

These results show that, in general, mice showed improvement in performance over continued testing on each replication of the T-maze alternation tests. The mice were relatively old (58 weeks) by the end of the study but overall levels of performance on the T-maze were not significantly different from one replication to the next, so statistically age has made no difference to performance on the T-maze. In any event, a general effect of age on learning ability could not account for the differences observed between the groups at any particular point in time. Mice housed in dyads that were identified as having a higher severity of aggression showed a clear learning deficit, compared to mice that remained paired and those that were separated as an experimental manipulation. Cognitive impairment in the aggressive separated group was found to be persistent, as it was evident across both replications of testing. Furthermore this effect of housing was also found to be dependent upon social status as whilst dominant mice performance did not differ between the 3 groups there was a deficit in subordinates from the aggression-separated group compared to the continuously paired and experimentally separated mice.

The performance deficit shown by subordinate mice in the aggression separated group, could, in principle be attributed to a proactive effect (on spatial learning) or a retroactive effect of social stress (on consolidation). However, the deficit continued 13 weeks after separation so there was no evidence for a retroactive component that could be improved by stopping aggressive encounters in the home cage (cf. Barnard & Luo, 2002). It has been proposed that the effects of a single experience of social defeat may cause behavioural or physical disruptions that can last up to a period of weeks (Koolhaas, Meerlo, De Boer, Strubbe, & Bohus, 1997), and this may explain why cognitive impairment persisted in these subordinates even after separation

Table 1
Mean corticosterone levels (mg/mol creatinine), together with standard errors, taken at two time points, pre- and post-initial pairing

Social status	Housing condition	Assay	Mean	Standard error
Dominant	Paired	Pre-pairing	14.61	3.48
		Post-pairing	9.33	1.75
	Aggression separated	Pre-pairing	12.69	4.26
		Post-pairing	15.29	2.14
	Experimentally separated	Pre-pairing	6.35	4.26
		Post-pairing	8.89	2.14
Subordinate	Paired	Pre-pairing	13.74	3.81
		Post-pairing	10.76	1.92
	Aggression separated	Pre-pairing	29.5	4.26
		Post-pairing	11.45	2.14
	Experimentally separated	Pre-pairing	15.12	3.81
		Post-pairing	15.71	1.92

These data are shown by subsequent experimental groups for mice that developed social status after pairing (into dominant versus subordinate members of each dyad). Housing condition refers to 3 later groupings in replication 2 of T-maze testing (paired, experimentally separated and aggression separated).

had ceased aggressive encounters. Corticosterone results showed that overall the aggressive separated group had higher pre-existing levels compared to the less aggressive groups. Increased levels of corticosterone have been shown to impair learning, especially when elevated over a long period (De Kloet, Oitzl, & Joëls, 1999).

However, in the present study, the initially very high levels of corticosterone (measured prior to any exposure to their cage mate) in the aggression separated group of mice which would become subordinate, dropped on pairing, rendering an account in terms of consistently elevated levels of corticosterone implausible. This pattern of effects suggests the possibility that, rather than some consistent difference in levels, there was a difference in the stress responsiveness of the mice which turned out to be particularly subject to social defeat (in pairings that polarised to the extent that they had to be separated). The deficit is pre-existing rather than a response to repeated attack because it was seen pre- rather than post-pairing. The association with performance on the learning task could arise if natural selection for spatial ability also results in selective pressure for dominance-related traits: both are heritable and mouse strains that are good at spatial learning are typically non-aggressive (Spritzer et al., 2004). We suggest that high corticosterone, measured non-invasively in urine, could in principle be used to predict the likelihood of long term learning deficits in association with, but not directly caused by, social subordination.

Learning impairments in subordinates have hitherto been presumed attributable to the animals' exposure to, and relative standing within, the social group (Barnard & Luo, 2002; Drea & Wallen, 1999; Nicol & Pope, 1999). This leads to the unproven assumption that the negative effects of social subordination should be remedied by separating the animals concerned. However, our results show that this is not necessarily the case. This has implications for our understanding of the causes and effects of subordinate status in other species too. The learning deficit that we measured persisted and we furthermore suggest the use of corticosterone measures as a way to identify those individuals likely to suffer deficits, irrespective of their experience of social defeat.

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