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Short communication

Do social statuses affect the startle reflex in male mice?

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HIGHLIGHTS

- Stable dominance/subordination relationships are obtained in pairs of male NMRI mice.
- We assess startle reflex and its prepulse inhibition in dominant and subordinate male mice.
- No significant differences were found in the startle response or PPI.

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ABSTRACT

Usual housing conditions lead to dominance hierarchy forming between male mice. The situation produces physiological and behavioural differences between dominants and subordinates. The goal of the present study was to assess stress responses, and possible changes in prepulse inhibition (PPI) of the startle reflex in dominant and subordinate male mice. Three weeks of daily social interactions led to stable aggressive dominance in 11 pairs of male NMRI mice. Stress levels were assessed by measuring faecal corticosterone metabolites (FCM), a non-invasive technique for monitoring hormonal changes in response to specific situations, with repeated sampling of each animal. The analysis of FCM levels showed greater stress in subordinate males at the beginning of the experiment, as the hierarchy was being established, but by the end of the experiment, FCM levels were reduced and similar in both dominants and subordinates. No significant differences were found in the startle response or PPI.

The startle reflex is a contraction of the skeletal musculature in response to an intense sensory stimulus. The startle reflex can be modulated by changes in the emotional state of the organism [13,14] and is enhanced in humans suffering from anxiety disorders [10]. Sensorimotor gating of the startle reflex happens when the reflex is inhibited by a weak 'prepulse' that occurs prior to the startling stimulus. Prepulse inhibition (PPI) of the startle reflex provides a measure of sensorimotor gating, and deficient PPI has been reported in several neuropsychiatric disorders [5]. In mice, PPI defects are investigated in neuropsychiatric disease models that apply environmental stressors [22]. Persistent PPI deficits are found in mice subjected to repeated social test when they were individually housed, while group housing prevented PPI deficits [1]. PPI deficits were also induced by isolation rearing in mutant and inbred mice [8,20,27]. In these studies, however, isolated animals were compared to grouped animals without considering a possible effect of social status on startle reflex and PPI in the grouped animals. In laboratory conditions, male mice are often group-housed, that may result in aggression problems, but a hierarchy of dominance is often established in which subordinate postures can prevent aggression by the dominant mouse [15]. An ethologically oriented model of chronic psychosocial stress was proposed [2,3] that offers the opportunity to investigate whether social status (being dominant or subordinate) is a factor affecting physiological and behavioural responses in male mice. In the present study, we used the same paradigm to investigate the possible effect of stable social statuses on the acoustic startle reflex and its prepulse inhibition. The outbred NMRI strain was chosen because of its proven responsiveness to social defeat [12].

Twenty-eight male NMRI mice aged 9 weeks (supplied by IFFA CREDO) were divided into 6 groups of 4 or 5 males. After 2 weeks, males from different groups were paired and placed in 1 of 14 clear plastic boxes (38 cm long, 30 cm wide and 15 cm high) divided in half by a perforated transparent partition allowing visual and olfactory contact but preventing any body contact. Each compartment had its own supply of food and drink. The partition allowed

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us also to separately collect the faeces produced by each male of the pair. Each compartment floor was covered with an absorbent mat made of polypropylene (POLLUSORB, Haleco, France) that was replaced by a clean mat 24 h before each faeces collection. Animal use procedures were carried out in conformity with the European Community Council directive of 24 November 1986 (86/609/EEC).

Our procedure is a modified version of the chronic psychosocial stress procedure [2], and allows stable dominance/subordination relationships to be established. The two males of each pair were kept each in its own compartment with the partition in place for 24 h before the first physical contact. Faeces of day 0 were collected to assess the level of stress before the first encounter and the first probable fight. Five times a week (starting on day 1) the partition wall was removed for 10 min to allow agonistic interactions. The testing conditions were run for 3 weeks and each pair had a total of 15 encounters. Dominant and subordinate roles were observed and recorded on the basis of the postures displayed [26]. One member of the pair was said to be dominant if it attacked, bit and chased the other male, which was ranked as subordinate, displaying defensive behaviour (sideways and upright postures, withdrawal, lying on the back and freezing). Encounters were video recorded and the duration of aggressive behavioural interactions (i.e., interactions involving attacks, bites, and chases) were computed. If any attack lasted longer than 3 min, the partition was lowered to put an end to the encounter. For the remainder of the day, the transparent barrier kept the animals apart; they could see and smell each other, but could have no physical interaction. After each daily encounter the cage floor was cleaned, and the 24 h faeces following the encounter were collected on days 1 (first encounter), 3, 7, 10, 14, and 17, in order to control the stress level during the establishment of the hierarchical relationships. The most important glucocorticoid triggered by stressful situations in mice is corticosterone [23], which we assessed by measuring faecal corticosterone metabolites (FCM), using an enzyme-immunoassay. The assays were performed at the Institute of Biochemistry at the Veterinary University of Vienna, Austria. Methodological details for the enzyme-immunoassay are as described in Touma et al. [24,25].

The last encounter took place on day 21. At the end of the 3-week period of daily encounters, a stable and clear pattern of dominance emerged with a clear dominance hierarchy in 11 of the 14 pairs of males. In one pair, both males were aggressive throughout the three weeks, with no distinguishable dominant/subordinate hierarchy emerging. In two other pairs, one male spent much of the time grooming the other, and few aggressive behaviour patterns were recorded over the last week, preventing to reliably assign the social statuses to the males. These three pairs were excluded from the analysis, which focused on the remaining 11 pairs.

On day 22, acoustic startle reflex and PPI were assessed for both dominant and subordinate mice. The startle amplitude exhibited in response to an acoustic stimulation was measured using a MED Associates Startle Reflex System (MED Associates, Inc., St. Albans, VT). Assessment of PPI followed a modified version of procedures described by Salinger et al. [21]. The mice were placed in a mouse holder mounted on a linear load cell sensor platform located in a foam-lined sound-proof cubicle. After 5-min acclimation, mice were tested with 50 trials over 10 successive sessions. Each session consisted of five types of trials presented in pseudo-random order in 10 blocks; three of the five had a 20-ms prepulse stimulus (72-, 76-, 84-dB white noise) occurring 100 ms before the onset of the 40 ms, 120-dB white-noise startle stimulus. The fourth type of trial had the startle stimulus alone, while the fifth, with background noise only (65 dB), provided the baseline measurement for movement in the test chamber. The average inter-trial interval was 15 s (9–23 s range). The amplitude of the startle response was measured as the maximum startle amplitude (MSA) every 1 ms for 65 ms starting with the onset of the startle

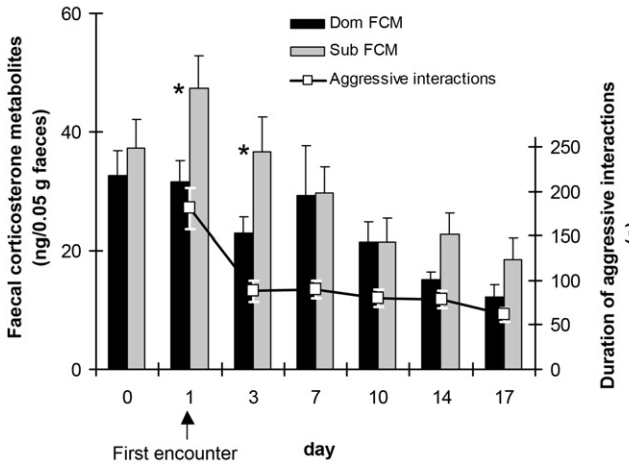

Fig. 1. Faecal corticosterone metabolites (FCM) concentrations and duration of aggressive interactions between dominant and subordinate male NMRI mice in 10-min encounters (mean ± SEM) during 3 weeks. The baseline values of FCM level on day 0 are the mean FCM level in the 24 h period before the first encounter. On days 1 (first encounter) and 3, the subordinate males had significantly higher FCM levels than dominant males (*$p < 0.05$). The high rate of FCM on day 0, i.e. before the first encounter, may be attributed to the mice being placed in new experimental conditions. FCM level increased significantly between day 0 and day 1 in subordinates ($p = 0.01$), indicating that stress increased immediately after the social defeat. There was no significant change in FCM after the first encounter in dominant mice. FCM significantly decreases with time, both in dominants and subordinates. Duration of aggressive interactions significantly decreased from day 1 to day 17.

stimulus. The reduction of MSA following a prepulse, referred to as PPI, was calculated for the three prepulse intensities according to the following formula: %PPI = ((startle-alone MSA − prepulse trial MSA)/startle-alone MSA) × 100. The reduction in MSA was interpreted as startle-response habituation (SRH), assessed by the change in responses to 10 startle stimuli (120 dB white noise, 40 ms) in the trials with startle stimuli alone. SRH was calculated as the percentage change in the amplitude of the startle response, according to the following formula: %SRH = ((MSA of last five trials − MSA of first five trials)/MSA of first five trials) × 100.

Because durations of aggressive interactions did not meet normality criteria, repeated measures ANOVA on ranks (Friedman test, followed by Newman–Keuls tests when appropriate) was used to evaluate significant changes over time. Changes in faecal corticosterone metabolites were analysed by two-way repeated measures ANOVA with “days” as the within-subject factor and “social status” as the between-group factor. When appropriate, post hoc contrasts by *t*-tests with a Bonferroni correction were performed. FCM levels in dominant and subordinate mice at day 0 and day 1 were compared by Student’s *t*-tests for paired samples, as the data passed the normality test. Changes in PPI levels in relation to the prepulse intensity were analysed using repeated measures ANOVA with “prepulse intensities” as the within-subject factor and “social status” as the between-group factor. Post hoc *t*-tests with a Bonferroni correction were performed. Effects of social status on MSA, SRH, and PPI were analysed by *t*-testing or Mann–Whitney testing when normality or equal variance assumptions were not reached.

A significant effect of day (chi-square = 21.2, df = 5, $p < 0.001$) on the duration of aggressive interactions was found. On day 1 (first encounter), the level of aggressive interactions was significantly higher than for any of the subsequent encounters ($p < 0.001$) (Fig. 1). On day 0, before the first encounter, the level of faecal corticosterone metabolites did not differ between dominant and subordinate mice ($p = 0.51$). There were no significant differences in the level of FCM between day 0 and day 1 in dominant mice ($p = 0.80$), and hence no stress effects in dominants after the first encounter. However, FCM level increased significantly after

Table 1
Maximum startle amplitude (MSA), and startle-response habituation (SRH) in dominant and subordinate male NMRI mice. Means ± S.E.M.

	Dominant	Subordinate	*p*
MSA	1890.11 ± 228.74	2154.75 ± 279.44	0.47
SRH (%)	19.87 ± 8.02	3.58 ± 7.59	0.16

the first encounter in subordinates ($p = 0.01$), indicating that stress increased immediately after the social defeat. A repeated measures ANOVA showed significant effects of social status ($F_{1,20} = 4.35$, $p = 0.05$), and day ($F_{5,100} = 9.45$, $p < 0.0001$) on FCM with an overall decrease in FCM level from day 1 to day 17 (Fig. 1). One-way ANOVAs confirmed a significant overall decrease in FCM level in dominants ($F_{5,50} = 3.14$, $p = 0.015$) and in subordinates ($F_{5,50} = 8.11$, $p < 0.0001$). The subordinate males had higher FCM levels than dominant males on day 1 ($p = 0.01$) and day 3 ($p = 0.03$). The levels of FCM were not different between dominant and subordinate mice on days 7, 10, 14, and 17 ($p = 0.94$, $p = 0.98$, $p = 0.24$, and $p = 0.33$, respectively).

There was no significant difference in the maximum startle amplitude between dominant and subordinate males (Table 1). Startle-response showed a 19.9% decrease in dominants over the test period, while for subordinates the decrease was only 3.6%, but the difference in startle reflex habituation was not significant (Table 1). A repeated measures ANOVA showed that PPI significantly increased when the prepulse intensity increased ($F_{2,36} = 52.6$; $p < 0.01$), and PPI was maximal at 84 dB prepulse (Fig. 2). Plappert et al. [19] already reported that PPI increased with increasing prepulse intensity in inbred mouse strains. PPI significantly increased in subordinates from 72 to 84 dB (from 72 dB to 76 dB: $p = 0.04$; from 76 dB to 84 dB: $p < 0.001$). In dominant males, PPI significantly increased from 76 to 84 dB only ($p < 0.01$); from 72 dB to 76 dB, the PPI increase was not significant ($p = 0.06$). For each prepulse level, the PPI tended to be greater in subordinates than in dominant males. However, there was no overall effect of social status on the PPI ($F_{1,20} = 1.16$; $p = 0.30$) (Fig. 2).

Studies of inbred strains by unpredictable chronic mild stress procedures subjecting mice to various stressors such as cage changes induce alterations in the concentration of faecal corticosterone metabolites [11]. Unpredictable mild stressors such as tilting the cage, changing the bedding, and reversing the light cycle have been used to induce anxiety and depressive-like behaviour in mice [18,28]. In the present study, the analysis of faecal corticosterone metabolites showed relatively high stress on day 0, i.e. before the first encounter, perhaps because the mice were placed in new experimental conditions (i.e., new cage and new social partner). FCM analysis showed a significant increase in stress in subordinate males after the first encounter, but the FCM level in subordinates went down to a level close to that of dominant males after a few days. Most models of chronic social stress have reported higher basal plasma corticosterone in subordinate male animals than in dominant and control animals [6,9,16]. However, experiments on rat colonies have shown that both dominant and subordinate males have higher basal plasma corticosterone levels than control non-socially housed males, suggesting that dominant animals also experience stress in social hierarchy environments [4,7,17]. In fact, an important factor affecting physiological and behavioural responses in grouped animals is whether a compatible group relationship and stable hierarchy can be established [3]. Experiments on male mice housed in groups of siblings showed that dominance status did not affect their reactions in anxiety/exploration tests, or change the hypothalamic-pituitary-adrenocortical (HPA) system activity and immune responsiveness [2]. It has been shown that an unpredictable chronic mild stress procedure decreases the amplitude of the startle reflex in male mice [18], and individual housing can also cause a deficit in PPI in mice [20,27]. Our data indicate that the social status of non-sibling pairs of male NMRI mice had no long-term effects on acoustic startle reflex or startle inhibition.

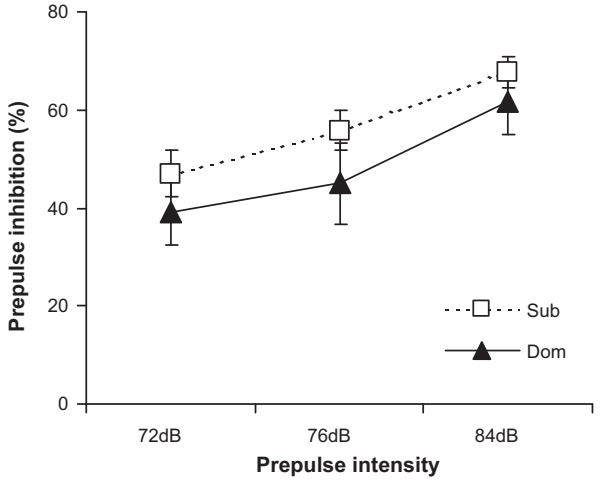

Fig. 2. Prepulse inhibition in dominant (Dom) and subordinate (Sub) male NMRI mice. Three 20-ms prepulse stimuli (72-, 76-, 84-dB white noise) were presented 100 ms before the onset of the 40-ms, 120-dB white-noise startle stimulus. Prepulse inhibition (PPI) is calculated as the percentage of the reduction of the maximum startle amplitude (MSA) for the prepulse trial over the MSA for the startle-alone trials: PPI = ((startle-alone MSA − prepulse trial MSA)/startle-alone MSA) × 100.

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