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Strain-Specific Aggressive Behavior of Male Mice Submitted to Different Husbandry Procedures

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Severe aggression within groups of male laboratory mice can cause serious welfare problems. Previous experiments have shown that the transfer of specific olfactory cues during cage cleaning and the provision of nesting material decrease aggression and stress in group-housed male mice. In this study, the combined effect of these husbandry procedures was tested for their long-term effect on aggression in two strains of male mice (BALB/c and CD-1). We used postcleaning aggressive behavior, wound counts, and testosterone levels as indicators of aggressiveness. Physiological responses to social challenge were investigated through urinary corticosterone and adrenal tyrosine-hydroxylase measurements. Furthermore, the aggression-modulating effects of two enrichment items (Shepherd-Shack/DesRes and PVC tube) were explored. Marked differences were found between the two strains. CD-1 mice were more aggressive, had higher testosterone levels but lower corticosterone levels, and had fewer wounds than BALB/c mice. However, in neither of the two strains was long-term enrichment with nesting material and its transfer after cage cleaning effective in lasting reduction of intermale aggression. This may be explained by the fact that aggression levels were generally low. It seems that housing mice in small, socially stable groups or keeping social disturbances to a minimum considerably modulates aggression in group-housed male mice. Mice of both strains housed in cages enriched with nesting material had lower urinary corticosterone levels than standard-housed mice. We therefore conclude that the long-term provision of nesting material, including the transfer of nesting material during cage cleaning, may enhance the welfare of laboratory mice. Aggr. Behav. 29:69–80, 2003.

Keywords: aggression; husbandry; environmental enrichment; male mice; welfare

INTRODUCTION

Aggressive behavior between male mice is mediated by social odor cues. These cues are involved in the advertisement of dominance over a defended territory as well as in the

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recognition and maintenance of social tolerance between familiar individuals [Hurst, 1990; Hurst et al., 1993; Van Loo et al., 2000]. Aggressive behavior in a socially stable group of mice is a natural part of a broader behavioral strategy of mice with a less flexible character [Benus et al., 1990a, 1990b, 1991; Sluyter et al., 1995]. In the wild, these mice will be fittest when population numbers are small. They exert despotic dominance through which they defend their territories against unfamiliar males, whereas they are relatively tolerant to familiar subordinate males [Crowcroft, 1966; Hurst et al., 1993; Mackintosh, 1970, 1973; Poole and Morgan, 1976]. When male mice are group housed in the laboratory, a certain level of aggression may thus be regarded as normal. Sometimes, however, aggression may rise to levels so high that subordinate animals become badly wounded and the mice need to be separated to avoid further serious welfare problems. These abnormal levels of aggression may have a genetic background or may be environmentally mediated. Strain-dependent differences in levels of aggressiveness have regularly been reported [Bisazza, 1982; Mondragón et al., 1987; Guillot and Chapouthier, 1998; Parmigiani et al., 1999]. Furthermore, environmentally induced disturbed social behavior may occur as a result of frustration or lack of control, situations that regularly occur in a laboratory environment [Broom and Johnson, 1993].

Measures currently taken to avoid the problem of excessive aggression in group-housed male mice are the use of females instead of males, the use of docile strains, and the use of individual housing. None of these measures can, as yet, be regarded as the optimal solution to the problem of excessive intermale aggression. It has frequently been shown that changes in husbandry procedures influence levels of aggression and stress in group-housed male laboratory mice. Cage cleaning, although essential for hygiene, disrupts odor cues and stimulates activity, which can have a significant effect on aggression in caged groups [Gray and Hurst, 1995]. Van Loo et al. [2000] found that transfer of odor cues from the nesting area decreased aggression compared with complete cage cleaning in group-housed male BALB/c mice, whereas transfer of sawdust soiled with urine or feces seemed to intensify aggression. The introduction of enrichment items into the cage might also affect intermale fighting. Besides an increase in the overall behavioral repertoire, several kinds of environmental enrichment have been shown to decrease intermale aggression [Ambrose and Morton, 2000; Armstrong et al., 1998; Ward et al., 1991]. Others seemed not to affect intermale aggression [Eskola and Kaliste-Korhonen, 1999] or even increased intermale aggression [Haemisch and Gärtner, 1994; Haemisch et al., 1994; McGregor and Ayling, 1990; McGregor et al., 1991; Van Loo et al., 2002]. Housing male BALB/c mice with nesting material significantly decreased the amount of intermale aggression [Van Loo et al., 2002]. Nesting material has furthermore been shown to be a highly preferred environmental enrichment in mice [Van de Weerd et al., 1997, 1998].

Recent studies by Van Loo et al. [2000, 2001, 2002] have focused on husbandry-induced changes in aggressive behavior in groups of mice. These studies were carried out with an inbred strain (BALB/c) known to be moderately aggressive [Eskola and Kaliste-Korhonen, 1999; Van Loo et al., 2000], and husbandry changes were applied for periods of 1 week. It is important to know whether effects of changes in the housing and husbandry can be extrapolated to other mouse strains and whether these changes will have the desired effect in the long term [Shepherdson et al., 1998]. The aim of the present study was to investigate the long-term effect of a combination of housing factors, previously found to decrease aggression, in males of both the inbred strain BALB/c and the outbred strain CD-1, the latter well known for its high levels of aggression [Parmigiani et al., 1999]. Parameters tested

were level of aggression after cage cleaning, number of wounds, testosterone, urinary corticosterone, and adrenal tyrosine-hydroxylase (TH) activity. The latter two measures reflect the HPA axis and sympathetic activation in response to challenges, respectively [Manser, 1992; Moberg and Mench, 2000].

METHODS

Animals and Husbandry

Sixty male mice of the BALB/cAnNCrlBR (BALB/c) and 60 male mice of the Swiss-derived CRL:CD-1(ICR)BR (CD-1) strain were used. At arrival, all animals were 6 weeks old. Per strain, the animals were randomly divided into 20 groups of three mice and housed in wire-topped clear perspex Makrolon® type II cages (375 cm^2, Tecniplast Milan, Italy) provided with 50 g of sawdust (Lignocel®3/4, Rettenmaier & Söhne, Ellwangen-Holzmühle, Germany). Half of the groups received nesting material (two Kleenex tissues, Kimberly-Clark Corporation®, Ede, the Netherlands) in addition to the usual bedding material (''enriched''). The other groups served as controls, without nesting material (''standard''). Pelleted food (RMH-B®, Hope Farms, Woerden, the Netherlands) and tap water were available ad libitum. The animal room had a controlled temperature (23-24°C), humidity ($60 \pm 5\%$), and ventilation (15-20 air changes/hr). The artificial light:dark cycle was 12:12, with lights on at 0700 hr. The mice were marked on the tail as well as on the fur with a black waterproof marker to enable individual identification. Marks were renewed weekly before cage cleaning. After arrival, the mice were allowed to adapt to their novel housing condition for 1 week. To monitor the general health status of the mice, mice were visually checked daily, wounds were counted twice weekly, and the mice were weighed. Food and water were weighed and refreshed once a week before cage cleaning.

Behavioral Data Collection and Analysis

Cages and wire-tops were cleaned weekly. For enriched cages, the nesting material was transferred from the dirty cage into the clean cage, and half of a new tissue was added to compensate for loss due to shredding or eating. At ages 9, 12, 15, 18, and 21 weeks, the behavior of the mice was recorded on videotape (Panasonic AG-6024-E; Matshusita Electric Industrial Co LTD, Osaka, Japan) for a period of 30 min immediately after cage cleaning.

The videotapes of behavior after cage cleaning were scored for latency until the first agonistic encounter, frequency and duration of agonistic encounters, frequency and duration of escalated encounters (i.e., encounters involving biting), and the total number of fights. The identities of the males involved in an encounter were recorded as well. Behaviors interpreted as agonistic included several offensive behaviors like fighting, biting, tail rattling, and chasing or vigorous sniffing of the head, tail, or genitals of the opponent and several defensive behaviors like upright and sideways defensive postures, flee, and active defense. The male showing the first agonistic approach was identified as the initiator of an encounter. When a male showed submissive behavior, the opponent was identified as the winner. In each group, dominant status was allocated to the animal initiating and winning the most encounters (dom). The two other animals were appointed a subordinate status (sub + when attacked most, sub − when attacked least).

Enrichment Pilot Test

To explore the aggression-mediating properties of two other enrichment devices, we conducted an additional test when the mice were 22 weeks of age. Immediately after cage cleaning, nesting material, if present, was removed from the cage and stored. Eight randomly chosen groups of both strains were provided with a ShepherdShack/DesRes (SSDR) house (Shepherd Specialty Papers, Kalamazoo, Mich) and six groups of each strain with a PVC tube (Ø 6 cm; length, 16 cm). The six remaining groups of each strain served as controls with no enrichment. For 30 min, all groups of mice were videotaped. After videotaping, the SSDR houses and the PVC tubes were removed and, if an enriched cage was involved, replaced by the old nesting material together with half of a new tissue. Agonistic encounters were scored as described in the "Behavioral Data Collection and Analysis" section.

Corticosterone, Testosterone, and TH Analysis

Urine samples were collected at ages 9, 12, 15, 18, and 21 weeks. Samples were taken noninvasively 3 to 4 days after cage cleaning between 9.00 and 10.00 hr to analyze urinary corticosterone and creatinine levels [method described by Dahlborn et al., 1996, and modified by Van Loo et al., 2001]. At age 22 weeks, the mice were decapitated simultaneously per group between 9.00 and 12.00 hr by three animal technicians, allowing blood collection without contamination of anesthetic compounds. Trunk blood was collected for testosterone analysis, and adrenals were dissected for TH analysis [method described by Van Loo et al., 2001].

Statistical Analysis

Data on testosterone values were analyzed using a general linear model for repeated measures with multiple comparisons, with age or status as the within-subjects factor and strain and treatment as between-subjects factors. The frequency of agonistic interactions as well as the amount and duration of the escalations were analyzed using an analysis of variance with negative binomial error, with time, strain, and treatment as between-subjects factors. The latency until first agonistic encounter as well as the duration of agonistic encounters, TH activity, and Co/Cr ratio were analyzed using a linear mixed-effects analysis, with as fixed factors treatment (all parameters), strain and time (latency, duration, Co/Cr ratio), cohort (TH), and status (Co/Cr ratio) and as random factors group (latency, duration, TH) or mouse number (Co/Cr ratio). The amount of fights and number of wounds were analyzed for the strains separately with the use of a Mann-Whitney U test, with treatment as the grouping variable. For CD-1 mice, the number of groups showing wounds was too small to perform inferential statistical analysis. To better conform to the normal distribution, several variables were log transformed. Correlations between the level of aggression (i.e., total frequency of agonistic encounters) and testosterone levels, TH activity, and Co/Cr ratio were analyzed using a partial Pearson's correlation, corrected for strain. Significant correlations were further investigated for strains and animals of different status separately. When multiple comparisons were made in any of the statistical analyses, Bonferroni correction was applied (i.e., P value multiplied by number of comparisons, indicated by P_B). To identify dominant and subordinate animals in each group, the level of individual aggressiveness was used. Five of the 40 groups showed no or hardly any aggression. As a result, the hierarchies of these groups could not be reliably evaluated. When comparisons

were made between dominant and subordinate mice, these groups were omitted from the analysis. All statistical tests were carried out with the aid of SPSS for MS Windows, Release 9.0 (SPSS Inc, Chicago, Ill) or S-plus 2000 Professional Release 2 (1988-1999, MathSoft, Inc.).

RESULTS

Behavior

No effects of housing condition on any of the postcleaning behavioral parameters could be established. For enriched-housed and standard-housed animals, CD-1 mice showed more agonistic behavior than BALB/c mice after cage cleaning (Fig. 1). In both strains, frequency and duration of agonistic encounters decreased from age 9 to 12 weeks, after which it increased (Fig. 1, top, only frequency shown). Number and duration of escalations in CD-1 mice followed the same time pattern, while in BALB/c mice, both were near zero and increased after age 15 weeks (Fig. 1, middle, only duration of escalations shown). Strain and

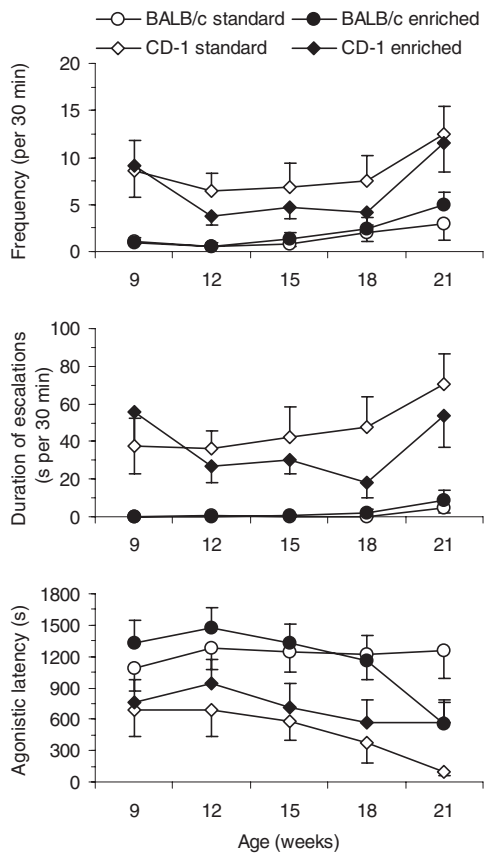

Fig. 1. Frequency of agonistic encounters (top), duration of escalated encounters (middle), and latency until first agonistic encounter (bottom) in 30 min after cage cleaning for BALB/c mice and CD-1 mice housed under standard or enriched conditions at five different ages. All data are presented as mean ± SEM.

time effects were significant for frequency of agonistic encounters, duration of agonistic encounters, as well as the duration of escalations (all: $P<.001$). For the frequency of escalations, only the strain effect was significant ($P<.001$). The latency time until first agonistic encounter showed an increase followed by a decrease when the mice grew older ($P<.01$) and was generally lower for CD-1 mice than for BALB/c mice ($P<.001$; Fig. 1, bottom). Fights were observed in only 7 BALB/c and 10 CD-1 groups. For BALB/c mice, the total number of fights observed during the whole experiment was six for the standard-housed and seven for the enriched-housed groups. For CD-1 mice, the total number of fights was 29 for the standard-housed and 16 for the enriched-housed groups.

Wounds

For mice of the BALB/c strain, a significant effect of the position in the dominance hierarchy was found ($P<.001$) (Table I). Dominant mice had the lowest number of wounds, whereas most-attacked subordinate (sub+) mice showed the largest number of wounds ($P_B<.001$). The average number of wounds of least-attacked subordinate (sub−) mice was significantly higher than that of dominant mice ($P_B<.001$) but lower than most-attacked subordinate mice (ns). No effect of housing condition was found. For CD-1 mice, wound count did not seem to be a good indication of level of aggressiveness: only 4 of 20 CD-1 groups contained wounded animals. In these four groups, the total number of wounds of the dominant mice varied between 0 and 5, of the most-attacked subordinate mice between 11 and 451, and of the least-attacked subordinate mice between 1 and 233. Three of the groups were housed under standard conditions, 1 under enriched conditions.

Enrichment Pilot Test

Behavioral data of the enrichment pilot test again revealed clear strain effects. CD-1 mice showed aggression faster and more often than BALB/c mice (latency: $P<.001$; Fig. 2, duration: $P<.01$). Furthermore, for both parameters, a time effect was present. Latency levels 1 week before the pilot test were generally lower and duration levels were generally higher than when the mice were subjected to the new conditions (latency: $P<.01$; Fig. 2, duration: $P<.001$). However, no significant differences were found between control groups and groups provided with either SSDR or PVC enrichment for any of the behavioral parameters measured.

TABLE I. Total Number Wounds Counted in BALB/c Mice (mean ± SEM [median])

	Status			
Housing	Dominant	Sub+	Sub−	Unknown
Standard	3.0 ± 1.1^{a} [3]	28.9 ± 5.6^{b} [25.5]	20.5 ± 5.5^{b} [17]	2.5 ± 1.4 [1]
Enriched	2.6 ± 1.1^{a} [1]	38.1 ± 13.0^{b} [30]	24.6 ± 12.4^{b} [17]	1.3 ± 1.3 [0]

$P<.001$.
[a–b]Different superscripts in a row indicate significant difference.

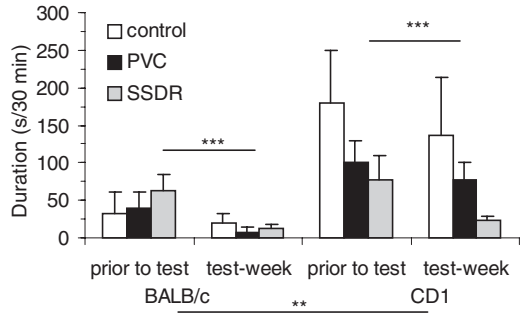

Fig. 2. Duration of agonistic encounters after cage cleaning (mean ± SEM) in BALB/c and CD-1 mice in the week prior to and immediately following the introduction of two new enrichment items. **$P < .01$; ***$P < .001$.

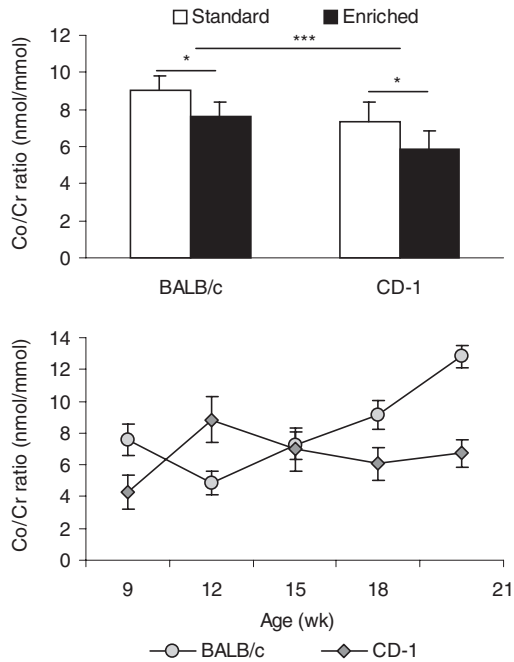

Fig. 3. Corticosterone-creatinine (Co/Cr) ratio (mean ± SEM) of BALB/c mice and CD-1 mice housed under standard or enriched conditions (a) and at five different ages, pooled for housing conditions (b). *$P < .05$; ***$P < .001$.

Urinary Corticosterone-Creatinine (Co/Cr) Ratios

For both strains, a significant housing effect on Co/Cr ratios was found (Fig. 3a, $P < .05$). Mice housed under enriched conditions showed a lower Co/Cr ratio than mice housed under standard conditions. Furthermore, a significant strain effect was apparent. CD-1 mice showed a significantly lower Co/Cr ratio than BALB/c mice (Fig. 3a, $P < .001$). Co/Cr ratios showed a significant time effect ($P < .001$), which differed between strains ($P < .05$, Fig. 3b: pooled for housing conditions). At age 9 weeks, the Co/Cr ratios of BALB/c mice were quite high, then decreased when the mice were 12 weeks old, and started to show an increase again

after age 15 weeks. CD-1 mice, on the contrary, showed lower Co/Cr ratios at age 9 weeks compared with BALB/c mice, followed by an increase at age 12 weeks, after which the Co/Cr ratios slightly decreased again. No effect of position in the dominance hierarchy on Co/Cr ratios was found. A significant positive correlation was found between aggression and mean Co/Cr ratios in dominant and most-attacked subordinate mice ($r = 0.4906$, $P < .01$ and $r = 0.3522$, $P < .05$, respectively). Closer scrutiny of the data revealed that most-attacked subordinate mice of the BALB/c strain and dominant mice of the CD-1 strain were mainly responsible for this correlation ($r = 0.558$ and $r = 0.556$, respectively, both: $P_B < .05$).

Testosterone Levels and TH Activity

Overall, CD-1 mice had higher testosterone levels than BALB/c mice ($P < .01$; geometric mean $\pm$ SEM of CD-1: 3.00 ± 0.57 ng/ml; BALB/c: 1.33 ± 0.29 ng/ml). No housing effects on testosterone levels were found, and dominance status did not have any significant effect on testosterone levels. The TH activity tended to differ for individuals with different positions in the dominance hierarchy (Table II, $P < .1$). Multiple comparisons revealed that dominant mice tended to have higher TH activity than least-attacked subordinate mice ($P_B < .1$). No effects of housing condition or strain were found. A significant positive correlation was found between TH activity and the level of aggression in a group ($P < .01$). Multiple comparisons showed that dominant and most-attacked subordinate CD-1 mice were mainly responsible for this correlation (Table II, $P_B < .01$, $P_B < .1$, respectively).

DISCUSSION

Housing Conditions and Aggression

The provision and transfer of nesting material neither reduced nor increased the level of intermale aggression in this study. This is contrary to results of previously performed studies by Van Loo et al. [2000, 2002] in which both the transfer of used nesting material and the provision of new nesting material clearly reduced the level of aggression. It is possible that

TABLE II. TH Activity (nmol/hr/adrenal pair; mean $\pm$ SEM)

		Dominance status				
House strair	Housing	Dominant	Sub+	Sub−	Unknown	
BALB/c	*Standard*	8.45 ± 1.10 a	8.46 ± 1.03	7.19 ± 0.73 b	(N = 8)	8.28 ± 1.14 (N = 6)
	Enriched	8.33 ± 1.70 a	8.21 ± 2.03	6.79 ± 0.73 b	(N = 9)	4.40 ± 0.54 (N = 3)
CD-1	*Standard*	8.38 ± 1.21 a	7.19 ± 0.90	6.95 ± 0.61 b	(N = 10)	—
	Enriched	8.44 ± 0.87 a	6.87 ± 1.20	7.30 ± 1.16 b	(N = 8)	5.18 ± 0.99 (N = 6)
	Correlation with aggression	$r = 0.649^{**}$	$r = 0.499^{*}$			

a-b, $^{*}P_B < .1$.
$^{**}P_B < .01$.

aggression-modulating factors acted on both the experimental and control groups. The most obvious candidate factors would be group size and level of disturbance. After all, none of the BALB/c or CD-1 groups needed to be separated during the experiment, since aggression levels remained tolerable. This is remarkable, since it is generally assumed that male mice of the CD-1 strain will kill each other when group housed [Mouse Genome Database, 2001]. Evidence exists that in small groups, as was the case in this experiment, the hierarchy is more stable than in groups of six to ten animals, i.e., group sizes often used in the laboratory [Butler, 1980; Poole and Morgan, 1973; Van Loo et al., 2001]. Furthermore, the mice in this experiment have hardly been subjected to stressful procedures. It is possible that in experiments in which mice are disturbed more frequently and more intensely, aggression will rise sooner and reach higher levels. Aggression toward cage mates due to frustration is a well-known phenomenon that has been shown in the domestic fowl [Duncan and Wood-Gush, 1971] and in rats and mice in which this phenomenon is used as a standard "shock-induced aggression" test [e.g., Driscoll et al., 1980; Pant and Nath, 1993]. It is possible that the provision and transfer of nesting material would have a more pronounced effect in groups of mice showing higher levels of aggression.

Another factor that may account for the discrepancy in results is that in the present study, mice were housed in the same conditions throughout the experiment, whereas in the former studies mice were intermittently subjected to different cleaning regimens or housing conditions. This intermittent character may have prolonged the novelty effect of the husbandry procedures, whereas in the present study the animals may have habituated to the nesting material and cage cleaning regimen. The introduction of a novel object has been shown to stimulate investigative or manipulative behavior that decreases as the animals habituate to the presence of the object or lose interest. Some degree of novelty can be sustained by adding materials or devices for short periods of time and changing them at intervals [Shepherdson et al., 1998]. The general decline in aggression when the mice were subjected to novel conditions in the enrichment pilot test supports this hypothesis. If nesting material, when it was still perceived as a novelty by the mice at 7 weeks, indeed affected intermale aggression, this effect may already have waned when behavioral recording started at age 9 weeks.

Social Status and Physiology

Housing condition did affect corticosterone levels. The lower corticosterone levels of enriched-housed mice found in this experiment are contrary to results of Haemisch and Gärtner [1994]. They found that enriched-housed mice showed increased levels of corticosterone, which they explained by their finding that mice in enriched cages were more aggressive and failed to maintain stable dominance relationships. An important difference between the latter and this experiment is the type of enrichment used. In a study by Van Loo et al. [2002], intermale aggression and corticosterone levels increased in mice housed in cages, structured with a shelter, comparable to the enrichment used by Haemisch and Gärtner [1994], whereas intermale aggression decreased in mice housed with nesting material. Social status and levels of aggression affected two neuroendocrine measures, corticosterone and TH activity. Both the urinary corticosterone levels and the TH activity of most-attacked subordinate mice and dominant mice were positively correlated to the level of aggression observed in the groups. It may be plausible to assume that the most-attacked or most-wounded animals would experience a more or less chronic intermittent social stress when

attacked by the dominant animal, leading to activation of both the HPA axis and sympathetic bodily response. Maintaining dominance through the initiation of aggression, on the other hand, also activates the sympathetic response and HPA axis activity [Haemisch and Gärtner, 1996; Maengwyn-Davies et al., 1973]. In concordance with the findings in dominant mice, the TH activity of dominant mice was higher than for least-attacked subordinate mice, whereas the TH activity of most-attacked subordinate mice was intermediate. Previous findings [Van Loo et al., 2001, 2002] are in agreement with these results. The correlation between corticosterone levels and aggression found for dominant mice may reflect the experience of chronic social stress associated with social instability. Higher levels of intermale aggression due to social instability have been reported previously [Poole and Morgan, 1973; Van Loo et al., 2001], as have elevated plasma corticosterone levels in dominant mice coinciding with increased levels of intermale aggression [Bronson, 1973; Haemisch et al., 1994; Van Loo et al., 2002].

Strain and Age Effects

The most pronounced differences in this study were found between the two strains tested. CD-1 mice were more aggressive than BALB/c mice, which is in concordance with these strains being referred to as highly and moderately aggressive, respectively [Eskola and Kaliste-Korhonen, 1999; Parmigiani et al., 1999; Van Loo et al., 2000]. The higher level of aggression of CD-1 mice was not only reflected in the behavioral data but also in the testosterone values. Testosterone mediates intermale aggression, and individuals with high testosterone levels are more aggressive [Dessi-Fulgheri et al., 1976; Zielinski and Vandenbergh, 1993]. However, the number of animals injured by fighting was higher in BALB/c mice compared with CD-1 mice, although the few CD-1 mice with wounds were more seriously injured on their backs and genitals. BALB/c mice had smaller wounds on their tails and backs, which may indicate a difference in offense or defense strategy. Another possible explanation may be a difference in fur thickness. Although we have not determined the exact fur thickness in both strains, the fur of CD-1 mice is thicker than that of BALB/c mice. Despite the overall lower levels of aggression in groups of BALB/c mice, they had higher urinary corticosterone levels than CD-1 mice. This difference may be due to strain-dependent susceptibility to social stress. In accordance with this, Kopp et al. [1999] showed that mice of the BALB/c strain are particularly susceptible to chronic stress exposure compared with several other inbred mouse strains.

After a minor decrease in aggression at the start of the experiment, intermale aggression increased with age. Goldsmith et al. [1978] and Van Loo et al. [2001, 2002] have reported a similar development of aggression: In newly formed groups, agonistic encounters are necessary to establish a dominance hierarchy. After a dominance hierarchy has been formed, the mice will live in a relatively stable environment for a while, after which aggression will increase again with age. For BALB/c mice, the curve of the corticosterone levels matched this development in aggression. This is in concordance with the finding that corticosterone levels and aggression were positively correlated and with previous and other studies [Bronson, 1973; Goldsmith et al., 1978; Van Loo et al., 2001, 2002]. For CD-1 mice, on the other hand, the aggression and corticosterone curves did not match as perfectly. Instead, levels increased from age 9 to 12 weeks and declined slightly afterwards. A reason for this is difficult to give.

Conclusion and Recommendations

The most pronounced effects in physiological as well as behavioral parameters were mainly found between the strains, with CD-1 being the more aggressive strain. Long-term enrichment with nesting material and the repeated transfer of nesting material when cleaning the cages did not affect intermale aggression. Remarkably, though, aggression never rose to unacceptable levels, implicating that forming small, stable groups and/or keeping experimental disturbances to a minimum may considerably modulate aggression in group-housed male mice. Furthermore, corticosterone levels are indicative of reduced stress levels in enriched conditions. Since the level of aggression is not negatively influenced by the provision and transfer of nesting material, the provision of nesting material and its transfer during cage cleaning is recommended for group-housed male laboratory mice.

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