# Social Rank in Male Mice and Adrenocortical Response to Open Field Exposure* (33622)

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When previously isolated mature male mice are placed together, fighting and the establishment of a social order often results. Physiological changes accompanying the behavioral responses associated with the social organization are reflected in altered adrenocortical and testicular endocrine activity. Changes in the concentration of brain catecholamines and in the toxicity to amphetamine indicate that gross central nervous system biochemical function is also affected (1–3). Subordinate males seem to be most adversely affected by grouping; for example, adrenal weights of subordinates are normally greater (4). The increased adrenocortical function in subordinates may be partially due to the effects of wounding, but it has also been shown that exposure, without contact, of a subordinate male to a trained fighter is a stimulus sufficient to cause a large increase in circulating adrenocortical steroids (5).

Based upon the above findings we hypothesized that subordinate and dominant males might also respond differently to nonsocial neurogenic stressors. The present study was designed to examine the plasma corticosterone levels in dominant and subordinate males following 10 min of exposure to a strange environment, i.e., an open field. Social rank was evaluated by observation and by correlating adrenal weight and concentrations of fructose in the seminal vesicles with the designated rank. Our findings show that there were only small differences in concentrations of circulating corticosterone in unstimulated animals of the various ranks. Exposure to an open field, however, resulted in large increases in circulating corticosterone on the part of subordinates, less so for dominants, and still less for unranked males (paired males in which rank was not discernible).

*Materials and Methods.* Male C57BL/6J mice were isolated at 21–25 days of age in 15 × 15 × 30-cm steel cages. At 75–80 days of age 160 males were paired and observed for 1 week for evidence of fighting and the establishment of social rank. Nine of the 80 pairs had one member either killed or too severely wounded to test, 16 pairs showed evidence of strong dominance–subordination relationship, i.e., wounding of a lesser degree on the rump and tail of the subordinate, while 55 pairs showed no marked evidence of fighting or hierarchy formation.

All 16 pairs of the category displaying strong rank but lesser wounds were chosen for this study while 12 pairs were chosen at random from the 55 unranked pairs. The experimental groups thus included 16 dominant, 16 subordinate, and 24 unranked males. Each of these groups was further subdivided into two treatments: those exposed to the open field and those not exposed to the open field (unhandled controls). Within each pair one animal, the unhandled control, was killed by decapitation immediately after removing its cage from the shelf. The remaining male in that cage was then placed in an

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TABLE I. Mean (± SE) Adrenal Gland Weights, Seminal Vesicle Weight, and Fructose Concentrations in Male Mice of Different Social Ranks.

| Social rank | No. of males | Adrenal wt. (mg) | Sem. ves. wt. (mg) | Sem. ves. fructose ($\mu$g/mg) | Sem. ves. fructose Total ($\mu$g) |
|---|---|---|---|---|---|
| Subordinate | 16 | 4.2 ± 0.12 | 52.3 ± 2.4 | 5.3 ± 0.2 | 280 ± 19 |
| Dominant | 16 | 3.1 ± 0.03 | 65.7 ± 3.7 | 5.7 ± 0.2 | 384 ± 38 |
| Unranked | 24 | 3.4 ± 0.05 | 66.3 ± 1.9 | 5.4 ± 0.2 | 364 ± 21 |

open field for 10 min prior to being decapitated. The open field used in this study was an enclosure with an open top 75× 75 cm painted flat grey and illuminated with a 100 W reflected bulb 75 cm above the floor. The order of treating dominant and subordinate males was randomized over all pairs. All animals were killed between 10 a.m. and 12 noon. A low volume noise generator was used in the mouse room to dampen response to normal laboratory sounds during the week of pretest pairing and during the testing period.

Body weight data were not obtained since the experimental procedure precluded handling mice before killing. As a consequence, adrenal gland weights are presented directly without correction for body size. The differences in adrenal gland size between males of different social ranks should be underestimated by this technique since the subordinates typically lose weight and show the greatest adrenal hypertrophy as a consequence of being defeated (8).

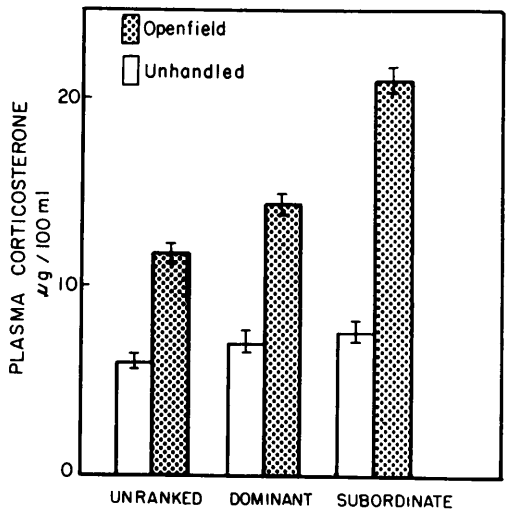


FIG. 1. Plasma corticosterone levels in unhandled and open field exposed dominant, subordinate, and unranked male C57BL/6J mice.

The corticosterone was determined fluorometrically after the method of Guillemin *et al.* (6). Whole blood was collected by drainage into heparinized tubes following decapitation and 0.2 ml of plasma was used for the analysis. The adrenals and seminal vesicles were removed, carefully dissected free of adhering connective tissue, and weighed. The secretions stored in the seminal vesicles was expressed by gentle pressure. The remainder of the gland was homogenized in water (10 mg/ml) and centrifuged at 10,000*g* for 15 min. A 0.75-ml aliquot of the supernatant was assayed for fructose (7).

*Results.* Responsivity to open field exposure, as assessed by amounts of circulating corticosterone, is shown in Fig. 1. There were no significant differences among the unhandled groups of males in plasma corticosterone concentrations, although dominant and, in particular, subordinate males tended to have higher plasma corticosterone concentrations than did unranked males. After 10 min of exposure to the open field, plasma of subordinate males had significantly more corticosterone than did the plasma of either the dominant or the unranked males ($p<0.01$ in both cases). Dominant males, in turn, had significantly higher concentrations than unranked males ($p<0.05$).

Adrenal glands of subordinate males were significantly heavier than those of either unranked or dominant males (Table I; $p<0.01$ in both cases). Unranked males had significantly heavier adrenal glands than dominant males ($p<0.01$). The average within-pair adrenal weight differences for unranked pairs was 0.3 ± 0.06 mg while the difference for

dominant–subordinate pairs was significantly greater, 1.2 ± 0.2 mg ($p < 0.01$).

Seminal vesicle weights and total seminal vesicle fructose levels were significantly lower in subordinate males than those of either unranked or dominant males (Table I; $p < 0.01$ in both cases). There were no significant differences between the weights or fructose content of the seminal vesicles of dominant and unranked males.

*Discussion.* No significant differences were noted in the concentration of circulating corticosterone between unstimulated animals of the three social categories even though weights of their adrenals differed. Previous reports dealing with exposure to trained fighting mice or with grouping of male mice or rats would indicate that such differences should have occurred (8–10). Exposure to an open field, however, made differences in social status readily apparent. Such exposure resulted in a greater increase in plasma corticosterone in subordinate males than it did in dominant males or in paired males which had not established social rank. The fact that dominant males had a greater response to open field exposure than did unranked males further suggests that the experience of being socially dominant can also lead to some degree of increased responsiveness to a strange environment. The data thus indicate that social subordination and, to a lesser extent, social dominance may lead to a greater responsiveness to nonsocial, neurogenic stressors. The most important implication of this finding for understanding population endocrinology is that studies of either grouping or social status involving adrenal function probably give minimal differences when examined in the generally unstimulating confines of a mouse box. Endocrine function of mice in natural populations, with their inherently greater and more variable levels of environmental stimulation, may be influenced more readily by their social status or by the frequency of social contacts than laboratory data have so far suggested.

While the study was not designed to pinpoint such a source, we can say that the differences in circulating corticosterone after open field exposure were not due to differences in amount of adrenal tissue and therefore must reflect increases in synthesis and/or secretion by the adrenal cortex.

The correlation between adrenal weight and the plasma corticosterone concentration following exposure to the open field was not significant ($r = 0.20$; $p > 70.10$). The lack of correlation was primarily a result of the dominant males having the smallest adrenals but a greater increase in plasma corticosterone than unranked males. Since it is hard to visualize such large, socially dependent, differences in rate of degradation of corticosteroids within 10 min, a more probable source of the observed difference is in the release of ACTH; a strong relationship between social status and central nervous system function is thus indicated (3). The absence of wounds on unranked pairs indicates that these males were cohabitating with a lower level of agonistic interaction than dominant–subordinate pairs. However, the greater adrenal gland weights of unranked males as compared with those of dominant males probably reflects some degree of fighting between unranked males when they were initially paired. Such a circumstance would be consistent with the explanation that social rank differences in corticosterone concentrations following open field exposure were a function of differences in central nervous system function and not the amount of adrenal gland tissue *per se*.

The finding that testicular androgen function, at least as assessed by fructose in the seminal vesicles, was reduced in subordinate males is similar to observations made in studies on the effects of grouping (1). The present study more clearly demonstrates that testicular function is decreased primarily in subordinate males and accompanies the increased adrenal activity. In fact, there is some suggestion that testicular functioning may be enhanced in dominant males which may be important if such males assume the major reproductive function in a population as suggested by Christian *et al.* (1). In this respect it is interesting to note the recent finding that LH concentration is actually

higher in the plasma and lower in the pituitaries of defeated males than in fighter males (9). The response to defeat may involve a release of both LH and ACTH suggesting that the ability of LH to stimulate testicular androgen secretion may be antagonized by increased circulating corticosterone since the seminal vesicles of subordinate mice were smaller and contained less fructose.

*Summary.* Plasma corticosterone levels were examined in socially dominant, subordinate, or unranked male mice taken directly from an undisturbed cage or following 10 min of a nonsocial neurogenic stress of exposure to an open field. There were no significant differences in plasma corticosterone levels among the social rank categories in undisturbed animals. Following open field exposure, subordinate males had the greatest levels of plasma corticosterone while dominant males had greater levels than unranked males. These data indicate that the process of social organization leads to an increase in responsiveness of male mice to a strange environment and under these circumstances the socially subordinate males are more responsive.

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