Endocrine and behavioral differences between dominant and subordinate male house mice housed in pairs*

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Adult male mice of proved fertility were housed in pairs for a period of 17 days. On the 17th day of such housing, the dominant and the subordinate member of each pair were identified by the degree of wounding and their behavior. Each mouse was then placed for 5 min in an aggression test situation with an immature "standard" opponent and its behavior observed. On the following day, the mice were killed and the weights of the bodies and a number of endocrine glands were obtained. Although there were no significant differences between dominant and subordinate individuals on any of the behavioral measures, the organ weight study confirmed that subordinate mice are characterized by high adrenocortical and low gonadal activity when compared to the dominant animals.

Briefly summarized, Christian's (1950) concept of the method of population limitation in rodent species (which is based on studies of house mice, housed at different densities) was that increasing density causes an increase in the incidence of fighting behavior, which causes consequent changes in adrenal and gonadal function. These endocrine changes, it was postulated, resulted in the increased mortality and reduced natality, characteristic of mice at high densities, which suppressed further population increase. This rather simple view of the role of agonistic behavior in the control of rodent population size, has since been complicated by the fact that aggressiveness of mice often appears to be suppressed in individuals housed at high densities as compared to isolated mice (Brain & Nowell, 1971a) and that hierarchy formation often results in the amount of fighting behavior being not directly related to density (see a recent review of the problem by Brain, 1971). I have recently (Brain, 1971) advanced another view of the possible involvement of fighting behavior and endocrine function in the mechanism of population limitation. It is noted that the normal habitat of the house mouse is not colonial. Mice live in small territories (Crowcroft & Rowe, 1963; Mackintosh, 1970) often described as "demes." These territories are held by dominant (on their area) males that do not normally tolerate other mature (especially unfamiliar) males in this region and, as dominant males are not subjected to the pronounced "stress" of defeat, they can be regarded as "behaviorally isolated." If such a view is correct, isolation (from other dominant male conspecifics), rather than a hierarchical social existence, is the normal mode of life of the dominant male mouse. The hierarchical existence of caged populations of male mice can be regarded as an artificial consequence, as subordinate individuals are unable to escape. This view is supported by the fact that mouse hierarchies have been described as nonlinear because they consist of a single dominant individual and a variable number (depending upon density) of subordinate mice. On this view, the endocrine differences between isolated and grouped male mice are regarded as being mainly a consequence of the effects of isolation (which equates with dominance and territory holding as such mice are never subjected to defeat within their cages), rather than being due solely to the effects of "crowding" being nonexistent. The difference between the two hypotheses is merely that in one case isolation is regarded as a rather artificially low density and in the other, it is regarded as the normal mode of existence for the dominant male mouse.

It has been previously shown that adult mice housed in pairs after a period of isolation show a more marked suppression of gonadal function and a more obvious increase in adrenocortical activity than mice housed in larger groups (Brain & Nowell, 1970a) and that isolation increases this response to pairing (Brain & Nowell, 1971b). Both these papers indicate that the effects of "social stress" are most marked in paired animals and, as the dominant and the subordinate individuals can be readily identified in such small groups, it was thought to be useful to study the effects of dominance and subordination on endocrine function in pairs and to see whether these changes reflected altered "aggressiveness" in the two categories.

METHODS

The adult male mice of the albino TT strain (originally obtained from A. Tuck and Sons Ltd., Essex, England) used in this study had been kept in relatively undisturbed conditions in a sealed room with a light regimen of 12 h on, 12 h off. They had been obtained from litters, born within a 5-day period, which had been culled on the day of birth to six and had then remained unhandled until weaning at 18-22 days of age, when they were segregated into single-sex groups of six. The animals used had been successfully mated at 9 weeks of age and at approximately 13 weeks of age, and 10-14 days following the birth of their litters, they were removed and housed in pairs in Makrolon cages measuring 25 x 21 x 9 cm.

On the 17th day of such housing, the dominant and the subordinate individual in each pair were identified by two independent methods: (1) The individual evidencing multiple scarring on the back, rump, tail, and genitals was judged to be subordinate. (2) The individual showing marked submissive behavior in response to spontaneous attacks from its cagemate on the mornings of the 14th, 15th, and 16th days of housing was identified as subordinate. In no case did the two methods produce different results. Cages in which one animal died before the end of the test period (21 such cages) were discounted, as were cages in which neither of the two occupants appeared to show any fighting behavior (3 such cages).

On the morning of the 17th day of housing, each mouse was placed, in turn, into the aggression test situation described in detail elsewhere (Brain & Nowell, 1970b) for a 5-min period with a 30-40-day-old naive male mouse from a group of six such animals (such a "standard" opponent is always subordinate in an encounter, so the amount of fighting could be expected to depend on the "aggressiveness" of the test animal alone).

A number of behavioral measures were obtained, and these included: (1) The composite aggression score (CAS). If the mouse bit its opponent, tail rattled, or elicited submissive behavior from the opponent, it was given an arbitrary score of "1." The composite aggression score was a simple summation of such scores. (2) The incidence of homosexual mounting behavior. This factor was recorded, as it was thought to be possible that sexual motivation would interfere with the manifestation of fighting behavior. (3) The accumulated

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Table 1
Mean Body and Relative Endocrine Weights for Subordinate and Dominant Male Mice Housed in Pairs (N = 20)

Category	Body Weight (g)	Relative Weight Left Adrenal (mg/100g)	Relative Weight Ventral Prostate (mg/100g)	Relative Weight Left Testis (mg/100g)	Relative Weight Preputial (mg/100g)	Relative Weight Preputial – Sebum (mg/100g)
Subordinate	36.65 ±0.85	9.06† ±0.29	53.05* ±4.48	336.55 ±9.12	137.20* ±10.41	95.45† ±7.98
Dominant	38.70 ±0.94	6.78 ±0.27	65.70 ±3.97	344.70 ±10.00	171.65 ±11.05	145.40 ±10.95

*$p < .05$ †$p < .001$—Difference between the two categories on the student t test

attacking time (AAT). The number of seconds actually spent biting the "standard" opponent were recorded. (4) The number of discrete bouts of attacking the "standard" opponent were recorded.

On the day following aggression testing, the mice were killed by rapid decapitation, and the weights of the body, the left adrenal gland, the left testis, the ventral prostate gland, and the left lobe of the preputial gland both before and after squeezing (i.e., minus the sebum content) were obtained. Each gland was cleared of adhering fat on saline-moistened filter paper by hand and weighed on an appropriate torsion balance.

RESULTS

The mean weights of the body and the mean relative weights of the recorded endocrine glands, together with the standard errors for each mean, are listed for subordinate and dominant mice in Table 1. Tables 2 and 3 list the individual aggression test data for subordinate and dominant mice, respectively.

There were no significant differences (on the Student t test) between mean body or mean relative testis weight in the two behavioral categories. However, on the basis of the Student t test, the subordinate animals proved to have relatively greater adrenal weights ($t = 5.755$, $p < .001$) and relatively smaller ventral prostate ($t = 2.113$, $p < .05$) and preputial gland weights ($t = 2.267$, $p < .05$) than their dominant counterparts. Removing the sebum increased the differential in preputial gland weight ($t = 3.694$, $p < .001$). None of the behavioral measures, however, proved to be significantly different in dominant and subordinate mice. The composite aggression scores and the incidences of mounting behavior in the two categories were contrasted by the comparison of the two proportions test (Johnson & Leone, 1964) and the accumulated attacking time and the number of attacks by the Mann-Whitney U test (Siegel, 1956).

DISCUSSION

The differential in adrenal weight between dominant and subordinate mice confirms an earlier finding by Davis & Christian (1957). Such a differential has been shown to be indicative of an increase in adrenal activity in the subordinate animal (Louch & Higginbotham, 1967). The lower relative weights of the sex accessory glands in the subordinate individuals confirms recent findings by Lloyd (1971) in groups of six male mice. Such a finding indicates that the subordinate animal shows a marked suppression in the production of testicular androgens compared to the dominant mouse (the weights of the sex accessories are an index of circulating androgen titres). There have been recent indications that this suppression of gonadal function may be a consequence of increased adrenal function (Desjardins & Ewing, 1971).

The lack of a marked difference in aggressive behavior between the two categories is, at first sight, surprising, as it is well-established that fighting behavior in rats and mice is strongly dependent upon androgens in the male. However, the fact that dominance/subordination polarity does not appear to markedly influence the amount of fighting in a neutral situation is less surprising when one considers that subordinate mice are

Table 2
Aggression Test Data for Subordinate Male Mice (N = 20)

Composite Aggression Score	Mounting Behavior	Accumulated Attacking Time (Sec)	Number of Attacks
0	0	0	0
0	0	0	0
0	0	0	0
3	0	26	4
3	0	8	1
1	Yes	0	0
1	Yes	0	0
3	0	17	3
0	Yes	0	0
0	0	0	0
0	0	0	0
1	0	0	0
0	0	0	0
3	0	62	16
0	0	0	0
0	0	0	0
0	0	0	0
0	0	0	0
3	0	21	5
3	0	89	14

Table 3
Aggression Test Data for Dominant Male Mice (N = 20)

Composite Aggression Score	Mounting Behavior	Accumulated Attacking Time (Sec)	Number of Attacks
0	0	0	0
0	0	0	0
0	0	0	0
0	Yes	0	0
0	Yes	0	0
1	Yes	0	0
0	0	0	0
1	0	0	0
0	0	0	0
0	0	0	0
0	Yes	0	0
3	0	45	7
0	Yes	0	0
0	Yes	0	0
3	0	9.5	6
0	Yes	0	0
3	0	22	11
3	0	4	2
3	0	35	18
0	0	0	0

quite capable of becoming dominant territory holders should a territory become vacant due to predation or disease and, consequently, it is not surprising that an established hierarchical response and the response in a neutral situation to an unfamiliar, nonaggressive individual should be completely independent.

It should be noted that, as regards the natural situation, the experimental design was artificial in that a highly inbred laboratory animal was used and the mice were forceably kept in contact by being caged together.

It is concluded that dominant mice are characterized in a paired situation by higher gonadal and lower adrenocortical activity than their subordinate counterparts but that these induced endocrine differences do not affect the incidence and intensity of the fighting response to a "standard" opponent in a neutral situation.

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