Effects of prior housing on endocrine responses to differential caging in male TO-strain mice

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Two possible reasons which may account for the inconsistent reports of adrenal and gonadal functioning in individually and group-housed mice were studied. The influence of the prior housing condition on the response to differential housing was examined. Continuously grouped or isolated mice were found to have lower corticosterone levels than those paired then isolated or isolated then paired. The influence of rehousing was apparent 32 days afterwards. The results support the suggestion that changing the housing condition of mice is stressful. The possible effect on endocrine function of different numbers of dominant and subordinate mice in different sized groups was examined. The findings did not support previous work which had found a similarity between dominant and isolated mice: it seems that the conditions used in particular experiments are critical to the results.

Studies which have assessed adrenal and gonadal functions in individually and group-housed rodents have produced inconsistent results. For example, when adrenocortical activity is measured, isolation has been reported to be both a high- and a low-stress condition (Brain, 1975). This paper examines one possible source of the variability in results, the influence of the prior housing on measures of hormonal activity. Some isolation/grouping comparisons start by individually housing all the mice and then allocating them to groups of different sizes, whereas others start with small groups and randomly allocate mice to the housing conditions. Prior housing could, consequently, be a source of the recorded variability when differentially housed mice are compared.

Another potential cause of discrepancies are the different social structures within groups. Varying group size may result in different ratios of dominant to subordinate animals, as the laboratory mouse often exhibits despotic dominance (Brain & Evans, 1975). Brain (1975), Brain and Benton (1977), and Welch and Welch (1971) have drawn attention to behavioral and physiological similarities between individually housed male mice and dominant males in a group. It has been suggested that "isolated" males may have affinities with territorial animals, in that they are not subjected to defeat in a specified area. Brain (1975) suggested that the "isolate's" behavioral responses are not really surprising when one considers that the laboratory mouse is probably derived from a territorial ancestor (Crowcroft & Rowe, 1963).

Thus, the present study was designed, primarily, to

investigate the effects of prior housing on the subsequent response to isolation and pairing, and secondly, to study hormonal differences in dominant and subordinate mice.

METHOD

The animals used were outbred albino TO-strain mice produced and housed under highly controlled conditions in Swansea (described by Goldsmith, Brain, & Benton, 1976). Standard opaque polypropylene mouse cages, measuring $30 \times 12 \times 11$ cm, were used throughout this study. The cages had wire tops containing an ad-lib supply of water and Oxoid 41B mouse pellets. The animals were housed in a quiet room which only contained mice of this strain and they were maintained on a reversed lighting schedule (white lights on 2200-1000 h).

The mice, which were initially housed in groups of 6 from weaning, were allocated, at 43 days of age, to individual cages (38 mice) or paired in identical cages (46 mice). Mice were not paired with their initial cagemates. After 32 days of differential housing, 20 initially singly-housed mice were paired and 24 (1 died) initially paired mice were allocated to the individual caging. Pairs were used in this study rather than larger groups because (1) such mice have been found by Brain and Nowell (1970) to show greater endocrine response to grouping than some larger groups, and (2) the dominant and subordinate animal can often be identified in these categories.

In summary, there were four experimental groups: (1) those individually housed throughout, (2) those paired then individually housed, (3) those paired throughout, and finally (4) those individually housed then paired. In addition, the dominant and subordinate animals were identified in Groups 3 and 4.

Behavioral Observations and Physiological Measures

For a week following the second period of housing, paired mice were observed in their undisturbed home cages for 10 min a day in order to determine social rank. Subordination was assessed both by

the occurrence of submissive behavior and the presence of superficial scars on the body at the end of the experiment. Social rank was determined in the continuous paired group at a comparable time. After a further 32 days, the mice were rapidly decapitated and plasma collected for fluorometric determination of "basal" corticosterone titer, using a modification of the method of Glick, von Redlich, and Levine (1964). Corrections were made for nonspecific fluorescence. Subsequently, the weights of the carcass, sex accessories, and adrenal were obtained. Organ weights were expressed relative to body weight (i.e., mg/100 g body weight). Results were subjected to one-way analyses of variance, and differences between pairs of categories were investigated using the Student *t* test.

RESULTS

The results for the four basic categories are presented in Table 1. Differential housing produced significant differences in basal corticosterone titers ($p < .01$). The two groups which stayed in their original housing condition had the lowest corticosterone titers. In the case of the mice paired throughout the experiment, the levels were significantly lower than in those mice isolated then paired ($p < .001$).

The relative preputial weights differed significantly between the four categories ($p < .05$). The mice individually housed throughout had significantly higher weights than those paired and then isolated ($p < .01$). A similar pattern of results was obtained with squeezed seminal vesicles, another index of gonadal function; there was an overall difference ($p < .05$), and mice individually housed throughout had heavier organ weights than those paired then isolated.

Table 2 presents the comparison between continuously individually housed mice and the dominants and subordinates from the undisturbed pairs. Only the measures of corticosterone were significantly different ($p < .05$). Dominants had significantly lower basal corticosterone levels than either isolates ($p < .05$) or subordinates ($p < .01$).

Table 3 includes the data from those animals whose housing was changed after 32 days. Both the unsqueezed ($p < .01$) and squeezed preputials ($p < .001$) were significantly different. The squeezed preputials of the dominants were significantly higher than both the individually housed ($p < .01$) and subordinate mice ($p < .01$).

Table 1

The Effect of Prior Housing on Adrenal and Gonadal Response to Differential Housing in Male Mice (Means and Standard Deviations)

	Individually Housed Throughout			Paired then Individually Housed			Paired Throughout			Individually Housed then Paired			df	F	p
	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n			
Body Weight	32.2	4.1	18	32.8	2.8	23	33.8	2.9	22	33.0	2.4	20	3,79	.41	n.s.
Relative Organ Weights															
Adrenal	8.4	2.9	18	7.5	2.6	23	7.3†	2.6	22	8.8	2.2	20	3,79	1.59	n.s.
Preputial	155.0*	70.1	18	110.5**	27.8	23	127.5	28.3	22	145.2	67.4	20	3,79	3.10	.05
Squeezed Preputial	95.1*	23.5	18	78.5	17.1	23	99.15	24.3	22	99.3	44.6	20	3,79	2.57	n.s.
Seminal Vesicle	313.3	100.8	18	312.2	59.2	23	302.2	58.7	22	287.2	77.5	20	3,79	.54	n.s.
Squeezed Seminal Vesicle	143.5*	52.1	18	108.2	33.7	23	135.5	39.8	22	114.5	25.9	20	3,79	4.00	.05
"Basal" Corticosterone	7.9	4.3	14	9.3	4.9	20	6.0††	2.1	20	10.2	2.8	15	3,67	4.77	.01

Note—*n* = sample size. Body weights are given in grams; relative organ weights are given in milligrams per 100 g; "basal" corticosterone levels are shown in micrograms per 100 ml.

*Continuously individually housed differs from paired then isolated, $p < .01$.

**Paired then isolated differs from isolated then paired, $p < .002$.

†Continuously paired differs from isolated then paired, $p < .05$.

††Continuously paired differs from isolated then paired, $p < .001$.

Table 2

Effect of Rank or Isolation on Adrenal and Gonadal Function in Male Mice Kept Continuously in the Same Housing Condition for 64 Days (Means and Standard Deviations)

	Individually Housed			Dominant			Subordinate			df	F	p
	Mean	SD	n	Mean	SD	n	Mean	SD	n			
Body Weight	32.2	4.1	18	34.8	2.8	11	32.7	2.6	11	2,37	1.82	n.s.
Relative Organ Weights												
Adrenal	8.4	2.9	18	6.6	2.8	11	8.1	2.2	11	2,37	1.57	n.s.
Preputial	155.0	70.1	18	138.6	28.1	11	116.4	24.9	11	2,37	1.93	n.s.
Squeezed Preputial	95.1	23.5	18	98.9	24.1	11	99.4	26.8	11	2,37	.49	n.s.
Seminal Vesicle	313.3	100.8	18	288.9	47.1	11	318.0	64.6	11	2,37	.44	n.s.
Squeezed Seminal Vesicle	143.5	52.1	18	132.2	39.7	11	138.8	41.7	11	2,37	.96	n.s.
"Basal" Corticosterone	7.9	4.3	14	4.7	2.1*	10	7.4	1.2	10	2,31	3.44	.05

Note—*n* = sample size. Body weights are given in grams; relative organ weights are given in milligrams per 100 g; basal corticosterone levels are shown in micrograms per 100 ml.

*Differs from subordinate category ($p < .01$) and individually housed ($p < .05$).

Table 3
Effect of Rank or Isolation of Adrenal and Gonadal Function in Male Mice (Means and Standard Deviations)
Switched to the Alternate Housing Condition After 32 Days

	Individually Housed			Dominant			Subordinate			df	F	p
	Mean	SD	n	Mean	SD	n	Mean	SD	n			
Body Weight	32.8	2.8	23	33.6	2.7	10	32.3	2.1	10	2,40	.11	n.s.
Relative Organ Weights												
Adrenal	7.5	2.6	23	8.6	2.6	10	8.9	1.7	10	2,40	1.64	n.s.
Preputial	110.5	27.8*	23	173.4	78.9	10	117.0	40.02	10	2,40	6.57	.01
Squeezed Preputial	78.5	17.1*	23	127.1	50.4	10	71.5	23.0*	10	2,40	11.83	.001
Seminal Vesicle	312.2	5.92	23	281.3	94.1	10	293.0	61.3	10	2,40	.79	n.s.
Squeezed Seminal Vesicle	108.1	33.7	23	121.3	32.1	10	107.2	16.3	10	2,40	.79	n.s.
"Basal" Corticosterone	9.3	4.9	20	8.9	2.9	8	11.5	2.2	9	2,34	1.14	n.s.

Note—*n* = sample size. Body weights are given in grams; relative organ weights are given in milligrams per 100 g; basal corticosterone levels are shown in micrograms per 100 ml. *Differs from dominant ($p < .01$).

When the data in Tables 2 and 3 were compared, both the dominant ($p < .01$) and subordinates ($p < .001$) had significantly lower corticosterone levels in the unchanged housing conditions. The dominants in continuously housed pairs had significantly lower adrenal weights ($p < .05$, one-tailed test) than those whose housing had been changed. The squeezed preputials ($p < .02$) and squeezed seminal vesicles ($p < .05$) of the subordinates in the changed housing conditions were significantly lower.

DISCUSSION

The results very clearly support the suggestion that changing the housing conditions of mice is stressful. The influence of rehousing was sufficient to produce higher basal corticosterone titers 32 days afterwards, even though the animal had not been subsequently subjected to additional stressful events. It is clearly important in conducting studies which examine the production of stress hormones to ensure that comparable housing conditions are used.

There is also evidence that changing housing decreases the weight of the sex accessory glands, there were significantly lower levels in both the isolates and subordinates. As the gonadal system is known to be suppressed by stress (Brain, 1972), this may again reflect the stress associated with a change in housing. The results confirm the report of Brain and Nowell (1971a), who found that pairing following a period of isolation was particularly stressful. It seems that a disturbance of housing conditions is more stressful than are the housing conditions themselves (at least at these densities). The workers who have discussed the "isolation stress" syndrome (Weltman, Sackler, Schwartz, & Owens, 1968), seem to have misnamed the phenomenon, it is not isolation as such which is stressful. However, the isolate's adrenocortical axis may be particularly reactive when subjected to stress (Benton, Goldsmith, Gamal-el-Din, Brain, & Hucklebridge, 1978).

An examination of the methodology used in particular studies may explain some of the inconsistencies in the reported results. For example, Brain and Nowell (1971b) reported lower levels of both basal and stress corticosterone levels in isolated than in grouped mice: this study combined three previously established smaller groups, and it could be expected that the formation of a dominance hierarchy would be especially stressful. Other studies which have used relatively unstressful designs have confirmed the present findings that there are no differences in the basal corticosterone levels in isolated and grouped mice (Benton et al., 1978; Goldsmith et al., 1976).

The significantly lower corticosterone levels in dominant mice compared with subordinate mice (Table 2) confirms a number of studies (Chapman, Desjardins, & Bronson, 1969; Henry, Ely, & Stephens, 1974; Louch & Higginbotham, 1967). The lack of a significant difference in the animals whose housing had been changed (Table 3) probably results from the stress in the dominants associated with a change in housing.

There is, however, some evidence that, in long-term groups, the plasma corticosterone in unstressed dominants does not differ from that in subordinates (Chapman et al., 1969; Henry et al., 1974).

A number of studies have reported that subordinates have heavier adrenals than dominant males (Brain, 1972; Brain & Nowell, 1971b; Chapman et al., 1969; Louch & Higginbotham, 1967; McKinney & Pasley, 1973; Welch & Welch, 1971). The present results, however, failed to produce a significant difference, although a similar trend was evident.

Dominants did, however, generally have heavier sex accessory glands than subordinates in the present study (also reported by Brain, 1972; Brain & Nowell, 1971a; Bronson, Stetson, & Stiff, 1973; Chapman et al., 1969). The weights of these glands are particularly influenced by testicular androgens. Consequently, the present results are consistent with those of McKinney and Desjardins (1973), who reported

that subordinate animals have lower circulating androgens and a lower rate of testosterone synthesis than dominants.

Brain and Benton (1977) have reviewed a range of both behavioral and physiological evidence that suggests that isolated male mice have many similarities to dominant animals. Benton et al. (1978) found that both isolates and dominants had heavy sex accessory glands when compared to grouped and subordinate animals.

The present results do not support the hypothesized similarity between dominants and isolates. The basal corticosterone levels of dominants from continuously housed pairs differed significantly from those of individually housed animals (Table 2). The preputial weights of isolates were significantly lower than those of dominants from groups which had changed housing conditions (Table 3). It seems that the parallels between dominant and isolated mice only hold under particular housing conditions. Those studies reporting a physiological similarity between dominants and isolates have tended to differentially house their animals for short periods starting at weaning and have preisolated grouped animals (Benton et al., 1978). These experimental conditions contrast with the present conditions, where differential housing took place at 43 days and longer periods of differential housing were used.

The present study provides another example of the importance of apparently minor changes in methodology on the analysis of the effects of isolating and grouping laboratory rodents. The results emphasize the need for careful, systematic investigation in this area.

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