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Effects of Social Isolation and Crowding upon Adrenocortical Reactivity and Behavior in the Rat

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The effects of social isolation and crowding on adrenocortical function and upon behavioral responsiveness to electric shock have been studied in male and female rats. All female experimental groups showed higher corticosterone levels and heavier adrenals than their male counterparts. The major effect of housing condition concerned the corticosterone response to stress, while basal hormone concentration was not modified. Socially housed rats showed a more intense adrenocortical response and also a greater behavioral reactivity to electric shock than the isolates.

Key words: Rat, Isolation, Crowding, Shock stress, Corticosterone, Behavior.

In a variety of mammalian species, high social density appears to induce a number of physiological alterations suggesting the activation of the General Adaptation Syndrome (9, 17, 28). From this survey, crowding could be considered as a model of chronic stress, the consequences of which must be reflected in certain modifications of the adaptation capacity of the animals subjected to this kind of social stress.

The possibility of considering high population density as a chronic stress has been previously taken into account (25); however, the data did not reveal clear differences between control (5 per cage) and overcrowed (20 per cage) male rats, concerning their emotional level, although heavier adrenals were found in the latter. On the other hand, while the time period during which male rats are kept in crowding conditions appears to be an important factor in modifying their Open Field (OF) behavior, as well as their ACTH response to noise (2, 3), suggesting an increased emotional reactivity, no significant effect of crowding was observed on serum corticosterone or on adrenal weight.

Social isolation was the other social

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treatment employed in this work, which has been found to induce several emotional alterations when animals were subjected to a variety of test including different stress intensities (4, 10, 11-13, 16, 22). Moreover, an adrenal cortex hyper-function has been observed after long periods of isolation (18). Nevertheless, the point of view that social isolation supposes a stressful situation is not supported by other results, at least in terms of adrenal function (e.g. 15).

The apparent contradiction among some of these data indicates that great complexity must exist in the physiological systems involved. A larger clarification of the role that the pituitary-adrenal axis plays in the emotional alterations induced by social isolation and crowding is needed, given the major importance of this axis in the stress response in mammals.

From a methodological point of view, comparison between resting corticosterone levels and the post-stress hormone levels should contribute to further information about the pituitary-adrenal axis functional flexibility in the animals subjected to different housing conditions.

Finally, both sexes were studied since the sexual dimorphism widely observed in the adrenocortical activity (8, 19-21, 27) and in several behavioral tests that include an emotional component (5-7, 17) suggests a possible different vulnerability of the two genders to social treatments.

Materials and Methods

Animals and Housing Conditions.— Male and female Sprague-Dawley rats were used. The animals were kept in a room at approximately 22 °C with a 12 h reversed light-dark cycle (2000-0800 h light on). Food and water were supplied *ad libitum*. Differential housing was introduced at weaning (22 days of age). Crowed rats (15 animals of the same gender per cage), and control rats (5 ± 1 animals of the same gender per cage) were housed in 48 × 24 × 15 cm standard laboratory cages. Social isolates were housed in 24×24×15 cm individual cages. Individually house rats could receive several stimuli (other than tactual) from the other animals living in the same room. Thus, isolation was considered as only «social».

The age of testing varied between 120 and 150 days.

Corticosterone assay.— Blood samples were obtained by decapitation of the animals at 120 days of age. Previously, they were submitted to an eight day habituation period according to GALLANT and BROWNIE's method (14).

Corticosterone circadian rhythm was taken into account so that blood samples were obtained in an appropriate phase of the photoperiod. Thus, the 4th hour after the onset of the dark phase (red light) was chosen, which corresponds to a median and relatively stable hormone level (1).

Decapitation was performed approximately 15 s after the animal was removed from the home cage. Heparin was used as an anticoagulant; blood was then centrifuged and the plasma obtained was stored at −25 °C until corticosterone concentrations were determined by Radioimmunoassay (RIA). Assay procedure was as follows: corticosterone (H^3) kits from Radioassay Systems Laboratories, Inc. were used. Plasma was diluted with buffer. Samples were incubated at 98 °C for 10 minutes to denature the corticosterone binding proteins in the rat plasma. The subsequent steps were those of a conventional RIA.

For the determination of the post-stress hormone levels, each subject was taken out of its home cage and carried in an individual cage to the experimental room. Then, it was placed in a shock box (which will be described later on) and submitted during one minute to 20 mA and 35 V footshocks delivered at 10 s intervals. Immediately afterwards, the animal was carried back to the laboratory house where it remained in an individual cage during a 15 min period. After this period, it was returned to the experimental room for decapitation.

Bilateral adrenalectomy.— Animals were operated on at about 150 days of age, under ether anesthesia. The adrenal glands were freed from fat and connective tissue and immediately weighed (fresh weight) (FW), and then kept at 40 °C during 48 h to obtain the dry weight (DW).

Adrenal weight relative to BW (FW/BW and DW/BW) were calculated for their subsequent statistical analysis.

Behavioral test.— Behavioral test took place in a 20×20×40 cm clear plastic wall box with a floor of parallel metal rods (each one with a diameter of 4 mm) connected to a DC power supply.

Each animal was tested two consecutive days, and each test lasted two minutes. Footshocks were delivered at 10 s intervals and the voltage was determined according to body weight, taking the 60 V assigned to the male control group as a reference. The following parameters were recorded as responses to stress: number of vocalizations (Voc); Jumping behavior (J): number of jumps, i.e. when the animal kept the four paws off the floor, as described by NISHIKAWA *et al.* (23); number of convulsions: brusque agitation body movements; number of Shudders: more attenuated movements; defecation score: number of fecal boluses deposited.

Statistical analysis was done taking the total values of the two days of testing.

Statistical analysis.— To study the effect of housing condition on behavioral (shock box) performance, a one way analysis of variance was used after square root transformation of data; *post hoc* individual comparisons of means were done by the Scheffé test. The same tests were used to analyze relative adrenal weights and plasma corticosterone concentrations. Bonferroni tests were employed to evaluate sex differences and the effect of stress upon corticosterone levels.

Results

Corticosterone assay and adrenal weight.— Females of the three experimental groups showed higher resting and post-stress corticosterone levels than their respective male counterparts ($p<0.01$).

The housing condition did not significantly modify basal corticosterone level, although a certain tendency in isolates to show increased resting hormone levels appears.

When each experimental group is compared to itself (basal level vs. post-stress level) (fig. 1), an increase in the cortico-

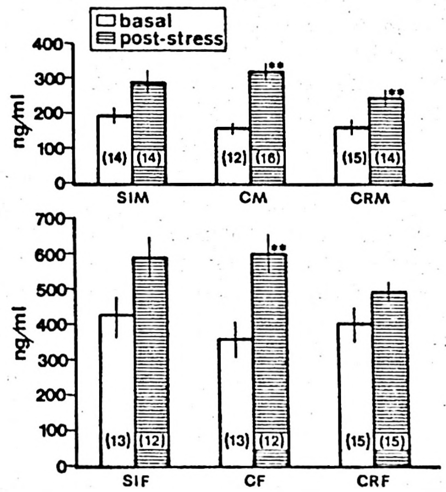

Fig. 1. *Plasma corticosterone levels of each experimental group.*
Values (mean ± SE) are compared to itself: basal level vs. post-stress level. SIM = isolated males; CM = control males; CRM = crowded males. SIF = isolated females; CF = control females; CRF = crowded females. In parenthesis number of animals. ** $p < 0.01$.

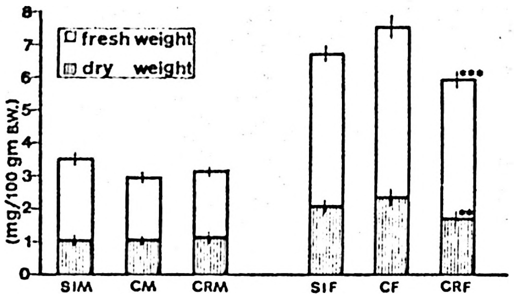

Fig. 2. *Adrenal weight of rats.*
SIM = isolated males, n = 13; CM = control males, n = 11; CRM = crowded males, n = 15; SIF = isolated females, n = 12; CF = control females, n = 13; CRF = crowded females, n = 9. Values are means ± SE. Comparisons between treated and control groups of the same gender.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.

sterone level in response to shock was observed in all groups; however, when male groups are considered, only socially housed animals (control and crowed males) showed a significant increase, while the isolates did not. Among female groups, the increase was significant only in the control animals.

All female experimental groups showed heavier adrenals (FW and DW) than their respective male counterparts.

The unique significant effect of housing condition, on this variable, concerned crowded females, which showed a lower adrenal weight when compared to the controls (fig. 2).

Behavioral test.— No significant sex difference was found between control groups in any of the parameters studied.

The unique significant effect of housing condition among male experimental groups concerned the isolates (table I) showing fewer vocalizations when compared to the controls. However, the repeated tendency of these control males to show higher values in all the variables (except in shudders), with respect to the treated animals (isolated and crowded males) reflects a greater reactivity of the control group in the present test.

Among female groups, control animals show a marked superiority in their responses to stress, particularly with respect to the isolates, so that the latter showed lower values in vocalizations, defecation, convulsions and jumps, while control and crowded females only differ significantly in vocalization rate. In contrast, isolated and crowded females showed more shudders than the control animals, although no statistical significance was reached (table I).

Discussion

Corticosterone assay and Adrenal weight.— Similarly to other studies (20), a higher basal corticosterone concentra-

Table I. *Behavioral test on rat.*
Voc = vocalizations; Def = defecation score; Conv = convulsions; J = jumps; Shudders. SIM = isolated males; n = 11; CM = control males; n = 10; CRM = crowded males; n = 14; SIF = isolated females; n = 23; CF = control females; n = 10; CRF = crowded females, n = 15. Values are means ± SE. Comparisons between treated and control groups of the same gender. * $p < 0.05$ ** $p < 0.01$ *** $p < 0.005$.

	Voc	Def	Conv	J	Shudders
SIM	17.5 ± 2.5*	6.2 ± 0.9	18 ± 2.4	12.6 ± 4.1	3.7 ± 1.2
CM	24.5 ± 0.8	8.2 ± 0.9	22.5 ± 1.4	15.6 ± 4.2	3 ± 1.3
CRM	20.2 ± 1.1	7.8 ± 0.5	20.9 ± 1.3	12.6 ± 3.6	2.5 ± 0.8
SIF	1 ± 0.6***	1.1 ± 0.3***	3.5 ± 0.8***	0.4 ± 0.1***	6.5 ± 0.7
CF	22.5 ± 1.5	7.1 ± 0.7	19.5 ± 2.1	13.3 ± 3.4	4.6 ± 1.5
CRF	12.7 ± 1.4***	5.6 ± 0.8	13.5 ± 1.5	11.9 ± 2.2	7.7 ± 1.1

tion was observed in control female rats when compared to the control males. However, the differences observed in the present study must not be considered as a rise of female corticosterone concentration during the period of handling prior to decapitation, as it was suggested (20), since the habituation period to which the animals were subjected makes this hypothesis unlikely in this work.

A sexual dimorphism in post-stress corticosterone levels has also been observed previously (19-21). Thus, post-stress plasma corticosterone levels have been reported as higher in female than in male rats following ether stress (20), psychological stress (21) and also after electric shock (19), with the latter results suggesting a greater adaptability of females' pituitary-adrenal axis response to this kind of harmful stimulation.

On the other hand, it has been well established that female rats show larger adrenals than male rats (27), as our results indicate, and a close relationship appears to exist between pituitary-adrenal and pituitary-gonadal axes, as several data indicate (8, 27).

The sex differences in adrenal function found in the present study, affect not only the control, but also the treated animals (isolated and crowded rats). Therefore, our social treatments have been, as it seems, insufficient to abolish those differences, and the same can be said concerning adrenal weights.

No significant effect of housing condition on resting corticosterone levels, or in general on adrenal weights, has been observed. Unlike other reports (18, 25, 26), these results suggest that basal adrenal activity is not modified by two extreme social conditions (social isolation and crowding). However, some methodological aspects might account for the discrepancies between the results, specially those concerning the number of animals housed per cage (25), a matter that suggests a certain gradation in the population density related to its effects on adrenal function.

The present results support other ones previously reported (15) concerning the absence of differences between individually and socially housed rats with regard to adrenal weights or basal corticosterone concentrations, employing an isolation treatment similar to that used in the present study.

The major housing condition effect found in this study refers to adrenal activity in response to stress, so that socially housed male rats (control and crowded animals) appear to respond to shock more intensely than the isolated males, while control females' response was more intense than that of the treated female rats. These results indicate that the major effects of this kind of environmental stress on the adrenal response, are due to changes in the pituitary-adrenal axis functional plasticity rather than to basal modifications of the adrenal activity *per se*, as previous results also suggest (24). The fact that socially housed animals show a more intense adrenal response than the individually housed rats, may represent a better adaptive mechanism to respond in a more suitable fashion to an intense stress.

Finally, the lower adrenal activity in response to stress showed by crowded females, and their lower adrenal weights when compared to the control females, are difficult to interpret; high population density might be thought to represent a type of chronic stress which alters the pituitary-adrenal axis balance, with the subsequent endocrine disturbances, gonadal axis being most probably involved.

Behavioral test.— The absence of differences between male and female control groups, might be interpreted as a homogenization of responses induced by intense stress (electric shock) in animals with a well adapted adrenocortical mechanism. Additionally, these results corroborate the validity of the voltages chosen for males and females in this test.

A greater reactivity was observed in this behavioral test in the control groups of the two genders when compared to the treated animals of the same sex, specially in the case of the respective isolates. These data parallel those which indicate a more intense short-term adrenocortical response to electric shock in the control groups, as pointed out in the previous section.

The utilization of electric shock (intense stress) both for endocrine and behavioral tests, makes it possible to compare the results obtained in both cases and to establish interesting parallels. In this sense, animals housed in standard population density conditions have shown greater adaptation ability in their behavioral responsiveness to this kind of stress, which manifests itself in their more intense responses that might be considered as «escape responses» from a harmful situation (23), specially when compared to the isolates.

The present results also indicate that the shudders represent a type of attenuated responses to electric shock, characteristic of those animals which do not show more intense psychomotor responses. Hence, individually housed rats tend to show increased values in that parameter (shudders) and minor values in the other variables studied in this test, with respect to the controls.

In general terms, the present results indicate that social isolation has been the more effective social treatment to affect the animals at the two levels considered (endocrine and behavioral).

Definitive conclusions about a possible different vulnerability to the social treatments used, in both sexes, cannot be deduced from the present data. Nevertheless, it may be useful to point out some differences affecting the adrenal system of crowded females and not that of crowded males, while among isolates the differences have been reflected at a behavioral level. Further research is needed in order to clarify these aspects.

Resumen

Se estudian los efectos del aislamiento social y del hacinamiento sobre la función adrenocortical y la respuesta comportamental al choque eléctrico en ratas machos y hembras. Todos los grupos experimentales de hembras muestran niveles más altos de corticosterona y cápsulas adrenales más pesadas que los correspondientes grupos de machos. El efecto más notable de los tratamientos sociales se observa en la respuesta de corticosterona al estrés, mientras que los niveles basales de hormona no son alterados significativamente. Las ratas criadas socialmente muestran una respuesta adrenocortical más intensa y una mayor reactividad comportamental al choque eléctrico que los animales aislados.

Palabras clave: Rata, Aislamiento, Hacinamiento, Choque eléctrico, Corticosterona, Comportamiento.

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