Post-weaning differential housing and testosterone secretion in male mice

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Summary. The effect of different densities of animals per cage on basal testosterone secretion and its response to some stimuli was studied in prepuberal male mice. Mice housed with 110 or 55 cm^2 of floor space per animal showed the same basal serum testosterone and hCG-induced testosterone release. Likewise, in a second experiment, the same basal serum testosterone and a similar response to acute noise stress were found in mice housed at 132, 66 or 22 cm^2 of floor space per animal. These results suggest that post-weaning crowding did not affect Leydig cell function in male mice.
Key words. Mice, male; housing density; testosterone secretion; crowding, post-weaning.

The effect of increased population density of endocrine function in rodents has been extensively studied[1-4]. It has been hypothetized that crowding could act as a stress stimulus to induce a general adaptation syndrome which, in turn, would inhibit reproductive function. Thus, crowding-induced inhibition of reproductive processes might act as a homeostatic mechanism controlling population size[5].

In spite of the abundant literature on the effect of crowding on reproductive function, there are few reports in which testosterone secretion was studied[6,7]. Moreover, most workers used mice individually housed in the post-weaning period. Further, they were maintained in single cages (control group) or rehoused in groups of different sizes. It is to be expected that such treatment would disrupt normal social development, and it could exacerbate the effect of crowding. The present work was done to show the effect of post-weaning differential housing on basal testosterone secretion and its response to some stimuli in male mice, since we have previously found that post-weaning crowding induced corticoadrenal hyperreactivity to stress in these animals (submitted).

Method. Male OF1 mice 20–22 days old were used. On arrival they were housed in groups of different sizes. The animals were maintained in controlled accommodation (light on 6.00–18.00, temperature 21 °C) and received food and water ad libitum.

In the first experiment the mice were placed in groups of 10 or 20 per cage (48 × 23 × 14 cm). After 29 days of differential housing, some were killed by decapitation within 2 min after being taken from their home cages. Other mice from both housing conditions were anesthetized with an i.p. injection of sodium pentobarbital (4 mg/100 g b.wt). 30 min later they were injected s.c. with saline or with 50 IU of human chorionic gonadotropin (hCG) and killed 30 min later.

In the second experiment mice were in groups of 4, 8 or 24 per cage (23 × 23 × 14 cm). 29 days after being placed in differential housing some were killed without any disturbance, and others exposed in 1 h of noise provoked by an alarm bell (85 dB). Adrenals and testes were removed, trimmed of fat and weighed.

Serum obtained from trunk blood was stored at −20 °C. Testosterone was analyzed by radioimmunoassay after extraction from serum with diethyl ether[8]. We used 1,2,6,7, ^{3}H-testosterone from Amersham (London) and testosterone-3-carboxymethyloxime-BSA antiserum purchased from Steranti

Table 1. Effect of increased density of animals into the cages on body weight and testis weight (mg/100 g b.wt) and serum testosterone (ng/ml)

Number of mice per cage	Floor space per mouse (cm^2)	Body weight (g)	Testes	Testosterone
10	110	44.0 ± 0.8 (25)	644 ± 29 (20)	3.80 ± 1.93 (10)
20	55	44.3 ± 0.9 (18)	626 ± 23 (20)	8.60 ± 2.33 (10)

Means ± SEM are represented. Number of animals per group in parentheses.

Table 2. Effect of increased density of animals in the cages on body weight, adrenal and testis weight (mg/100 g b.wt)

Number of mice per cage	Floor space per mouse (cm^2)	Body weight (g)	Adrenal	Testes	
4	132	34.35 ± 0.88 (10)	7.43 ± 1.33 (10)	930 ± 22	(10)
8	66	34.44 ± 1.05 (9)	7.60 ± 0.95 (10)	957 ± 45	(10)
24	22	31.75 ± 0.81*(10)	8.96 ± 0.69 (10)	933 ± 39	(10)

Means ± SEM are represented. Number of animals per group in parentheses. *p < 0.05 vs four animals per cage.

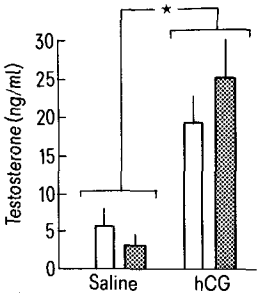

Figure 1. Testosterone response to hCG administration in male mice. Means and SEM are represented. Open bars indicate mice housed at 110 cm^2 of floor space per animal and closed bars mice housed at 55 cm^2 of floor space per animal. A significant effect of hCG administration but not of housing conditions was found. *p < 0.001 between groups. In each group n = 10.

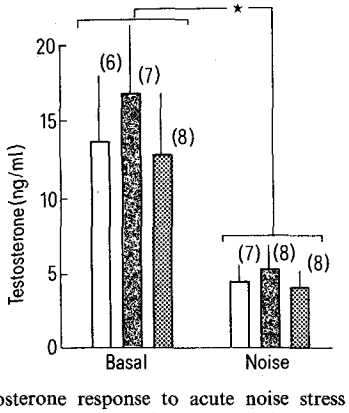

Figure 2. Testosterone response to acute noise stress in male mice. Means and SEM are represented. Open bars indicate mice housed at 132 cm^2, shaded bars, mice housed at 66 cm^2 and closed bars, mice housed at 22 cm^2 of floor space per animal. A significant effect of acute noise stress but not of housing conditions was found. *p < 0.01 between groups. Number of animals per group in parentheses.

Res. Ltd (London). Free and bound fractions were separated using charcoal-dextran. Intra- and inter-assay coefficients of variation were 8% and 17%, respectively.

Statistical analysis were performed with Student's t-test or with a two-way analysis of variance. All data were log, transformed before their statistical evaluation.

Results. The results of the first experiment are indicated in table 1. No effect of the number of animals per cage on body and testes weights was found. Likewise, both experimental groups showed the same basal serum testosterone. Analysis of variance revealed a significant effect of hCG administration ($p < 0.001$) but not of previous housing conditions on serum testosterone (fig. 1).

The results of the second experiment are depicted in table 2. Mice housed in groups of 24 per cage showed lower body weights than mice housed in groups of four per cage ($p < 0.05$). Neither adrenal nor testis weights were altered by housing conditions. Figure 2 shows the effect of acute noise on circulating testosterone in the three housing conditions. A significant effect of acute noise exposure ($p < 0.01$) but not of previous housing conditions on testosterone secretion was found.

Discussion. Our results indicate that the number of animals per cage during the post-weaning period did not alter either testis weight or testosterone secretion in male mice. In the first experiment both basal serum testosterone and its response to hCG were the same in both housing conditions. Therefore, no evidence for an altered responsiveness of Leydig cells to gonadotropin was found. These results were confirmed in the second experiment in which three different population densities were used. Neither basal testosterone nor its fall after acute noise exposure differed in the three experimental groups. Recently, it has been reported that the developmental pattern of testosterone secretion was altered in mice raised in high population densities; however, neither fertility nor the growth of the reproductive organs was affected at adulthood[7].

Although it is well accepted that crowding induces inhibition of reproductive processes and testosterone secretion[1-4], these effects are probably restricted to the post-puberal period in which dominance and hierarchies would be established. Thus, adult mice housed in groups together with non-familiar mice show frequent signs of physical injuries and these are exacerbated by increasing population densities. In contrast, we have not observed any sign of physical injuries in mice crowded in the post-weaning period.

Acute noise depressed serum testosterone in all mice irrespective of their housing conditions, suggesting that sensitivity to stress-induced inhibition of testosterone secretion was not modified by crowding. Hampered testosterone secretion after acute stress is a well known fact[9-13] and could be due to the inhibition of Leydig cells responsiveness to gonadotropin[14].

In conclusion, we have found that post-weaning crowding did not affect either the weight of adrenals and testes or serum testosterone. Using the same experimental design and animals of the same strain, age, and sex we have found that crowding neither modified adrenal weight and basal serum corticosterone nor increased fighting, in accordance with the results obtained in the present work. However, an increased corticosterone response to some additional acute stresses was found in crowded mice, indicating that post-weaning superpopulation affects some endocrine systems. A reevalution of the effects of crowding on the endocrine system is needed taken into account both the age at which crowding was started and the housing conditions before crowding.

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Inhibition of TSH-stimulated thyroid hormone release and potentiation of TRH-stimulated TSH release by indomethacin in perifusion systems of rat thyroids and pituitaries

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Summary. Using indomethacin (Ind), a prostaglandin synthesis inhibitor, in vivo experiments in rats and in vitro experiments with perifusion systems of rat thyroids and pituitaries were conducted. After 35 days of intragastric infusion of Ind, serum TSH levels were markedly increased, the thyroid was swollen and, as a consequence, T_3 and T_4 levels were normal. The T_3 release from perifused rat thyroids under continuous stimulation with 10 mU/ml TSH was inhibited significantly ($p < 0.01$) by 1.0×10^{-6} M Ind. On the other hand, the TSH release from perifused rat pituitaries under TRH stimulation was enhanced conspicuously by Ind. It was concluded that Ind decelerated thyroid hormone release from the thyroid and accelerated TSH release from the pituitary in perifusion systems.

Key words. Rat thyroid; rat pituitary; thyroid, rat; pituitary, rat; thyroid hormone release; indomethacin.

It has been suggested that prostaglandins (PGs) might mediate the effects of some hormones on the adenylate cyclase activity of their target organs[1,2]. It is well known that PGs in high concentration exist in the human thyroid[3], and that levels of endogenous PGs are lowered by PG synthesis inhibitors such as indomethacin (Ind) and aspirin[4-6]. However, the mechanisms of action of PGs on the thyroid function have not yet been clearly characterized.