Developmental effects of variable population density and dietary protein sufficiency upon morphology, neurochemistry, and behavior in the rat*

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Male Wistar rats were housed in groups of 2, 16, or 30 from 21 to 48 days of age. Each housing condition was factorially combined with one of three levels of dietary protein: 5%, 15%, or 25%. Body weight, brain weight, adrenal weight, and pituitary weight, as well as brain protein concentration, varied directly with protein level in the diet. Indices of neurotransmitter activity and nucleic acid concentrations were not differentially affected. The major effect of differential population density was associated with the combination of largest group size (30) and protein-enriched diet (25%). The large group size tended to minimize the effects of the protein-enriched diet. This combination also produced an increased responsiveness to extrinsic stress, as measured in the open field.

Although characteristically studied independently, early dietary deficiency and high population density are often correlated in their natural occurrence. Since there is some evidence that they affect, in part, the same physiological processes, it seems fruitful to investigate their possible interactive effects.

SUBJECTS

One hundred and seventy-four male Wistar rats, 21 days of age at the beginning of the experiment, were assigned randomly to one of nine treatment combinations, consisting of three groups sizes¹ (2, 16, or 30) factorially combined with three levels of protein-sufficient diet² (5%, 15%, or 25%).

APPARATUS

Experimental housing consisted of plywood cages with a mesh floor and top. Cage size was varied proportionally to the group size, with 20 sq in. allocated per animal.

Open-field testing utilized a 20 x 20 in. field painted flat black, with white lines dividing the floor into 4-in. squares. A 150-W bulb was centered 18 in. over the field.

Escape-avoidance conditioning utilized a one-way shuttlebox with a grid floor. The start compartment (9 x 12 x 12 in. high) was separated from the goalbox (15 x 12 x 12 in. high) by a guillotine door. A 20-W bulb, mounted in the rear wall of the startbox, was the CS. A 110-V scrambled shock to the grid floor was the UCS. CS-UCS and intertrial intervals were controlled by Hunter interval timers. Response latencies

were recorded on a Hunter .01-sec timer.

Organ weights were measured on an Ohaus scale calibrated in milligrams.

PROCEDURE

The Ss were housed in the assigned group-diet condition from 21 to 48 days of age. The diet, in the form of mash, was available ad lib for 45 min daily, with water available ad lib.

At 42 days of age, six Ss per group were selected randomly for behavioral testing in the open-field and escape-avoidance tasks. One 2-min trial in the open field was conducted on the first and sixth days of testing. On Days 2-5, each animal received 20 trials daily in the escape-avoidance task, using a CS-UCS interval of 5 sec and an ITI ranging from 30 to 60 sec. Throughout this testing period, all Ss continued to live under the experimental conditions.

At 48 days of age, six additional Ss were selected randomly for physiological assay. They were weighed and sacrificed, and brains, adrenals, and pituitaries were removed, weighed, and frozen for subsequent analysis. DNA, RNA, and brain protein determinations were performed on brain halves by differential extraction procedures

(Wannemacher, Banks, & Wumner, 1965). Norepinephrine and epinephrine concentrations were determined by a trihydroxyindole procedure, modified by microanalyses (Mead & Finger, 1961). AChE activity was estimated by a colorimetric method (Ellman, Courtney, Andres, & Featherstone, 1961).

Each of the dependent variables was analyzed as a 3 by 3 factorial analysis of variance.

RESULTS

Significant effects of diet were obtained for all morphological indices of growth. Significant differences were obtained for body weight ($F = 9.69$, $df = 2/45$, $p < .01$), adrenal weight ($F = 52.98$, $df = 2/45$, $p < .01$), brain weight ($F = 9.69$, $df = 2/45$, $p < .01$), and pituitary weight ($F = 13.82$, $df = 2/45$, $p < .01$). Table 1 summarizes these effects. It is apparent that, with the exception of pituitary weight, the effects were all due to decreased organ weights associated with protein deficiency. The protein-enriched diet only affected pituitary weights. There were no main effects of population density upon organ weights, but there was a significant interaction between population density and diet as they affected pituitary weights ($F = 3.01$, $df = 4/45$, $p < .05$). The pattern of means revealed that the increased mean pituitary weight associated with the protein-enriched diet was restricted to animals housed in groups of 2 or 16. Housing animals in groups of 30 eliminated the effects of extra protein upon the pituitary weights but did not interact with the effects of protein deficit.

The only significant chemical effect of diet was upon concentrations of brain protein ($F = 3.72$, $df = 2/45$, $p < .05$), with brain protein concentration varying directly with dietary protein levels. There were no effects of population density upon any of the neurochemical assays, nor were concentrations of DNA, RNA, epinephrine, or norepinephrine affected by diet. AChE activity was also unaffected by the experimental conditions.

Neither response latencies nor number of avoidance responses in the

Table 1
Mean Organ Weights as a Function of Dietary Protein

	Protein Level		
	5%	15%	25%
Body Weight (g)	163.83 (48)*	316.22	309.39 (2)
Brain Weight (g)	1.62 (9)	1.78	1.80 (1)
Pituitary Weight (mg)	5.26 (25)	7.01	8.12 (14)
Left Adrenal Weight (mg)	10.01 (41)	16.89	17.22 (2)

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*Denotes percentage difference from mean organ weight under 15% protein diet.

escape-avoidance task were differentially affected by diet or group size.

Significant interactive effects of diet, group size, and trials (pre- vs postconditioning) were obtained in open-field behavior, both for number of squares traversed ($F = 5.04$, $df = 4/45$, $p < .01$) and number of boluses ($F = 3.28$, $df = 4/45$, $p < .05$). The pattern of means indicate that none of the groups differed on either index on Trial 1 prior to the conditioning trials. Retesting following the 4 days of shock conditioning revealed slight drops in activity (and increases in defecation) for animals housed in groups of 2 or 16, whatever their dietary condition, and for animals housed in groups of 30 if they were receiving the intermediate level of dietary protein. Ss receiving a protein-enriched diet and housed in a group of 30 showed a marked reduction in activity, with a marked increase in defecation, across the two trials. Ss receiving a protein-deficient diet and housed in a group of 30 showed no changes across the two trials.

DISCUSSION

The general morphological effects of a protein-deficient diet upon young animals appears to be highly replicable. The percentage weight differences between normal and deficient protein levels in the present report correspond fairly closely to those previously reported (e.g., Lowry, Pond, Barnes, Krook, & Loosli, 1962; Howard & Granoff, 1968). The decreasing impact of dietary insufficiency upon weights of body, adrenals, pituitary, and brain, in that order, is consistent with Dobbing & Widdowen's (1965) report that dietary

insufficiency has its greatest impact upon those bodily structures which are simultaneously in their period of maximum growth rate. Presumably, the rank order of organs in terms of amount of impact of the dietary insufficiency would have been different if the diet had been manipulated at a different period of life, such as preweaning.

The failure to find differences in learning as a function of diet is reasonably consistent with the published literature (e.g., Howard & Granoff, 1968). It should be noted, however, that investigations of dietary effects upon learning have not utilized reward conditioning, presumably to avoid confounding of the experimental treatment with food reward, so that any conclusions regarding dietary effects upon learning, to date, must be restricted to aversive conditioning. There is virtually no published research on the effects of population density upon learning.

The major interactive effects between population density and diet appear to involve the treatment combination of the largest group with the protein-enriched diet. Animals in this combination appeared to be the most hyperreactive in the open field following escape-avoidance conditioning and failed to display extra pituitary weight, as did protein-enriched animals living in smaller groups.³ This hyperreactivity coupled with the effects on the pituitary suggests that the number of social contacts in the home cage resulted in animals which were possibly hyperstimulated. Such an interpretation, which will require systematic observations of behavior in the home cages for verification,

suggests that the impact of excessive population (e.g., Calhoun, 1962) may be greatest for the healthiest and most active animals.

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NOTES

1. Multiples of the smaller group sizes were employed to permit adequate sample sizes.
2. Protein test diets were commercially available diets from General Biochemicals, Chagrin Falls, Ohio.
3. Since different samples of animals were used in behavioral testing and physiological assays, the probability of this interactive combination being due to sampling error is unlikely.