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Choice of diets of differing caloric density by normal and hyperphagic rats

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When bilateral lesions are made in the ventromedial nuclei of the hypothalamus, striking and characteristic changes are produced. These effects, first demonstrated in the rat, include an enormous increase in food intake (hyperphagia) and the development of obesity (Hetherington & Ranson, 1939; Brobeck, Tepperman & Long, 1943). The hyperphagia occurs immediately after the operation, the rat eating large quantities of food as soon as it has recovered from the anaesthetic. When maximum obesity is reached food intake falls, and the excessive weight is maintained with a normal food intake. Similar lesions produce the same sort of changes in cats, dogs and monkeys. It is now generally believed that the ventromedial nuclei of the hypothalamus are involved in the maintenance of normal body-weight by the control of food intake.

In normal rats, food intake seems to be determined by calorie needs. Thus, Adolph (1947) has shown that, when rats were offered food diluted with kaolin or cellulose, they ate proportionately more food, so that their calorie intake remained constant. This 'eating for calories' was seen so long as the dilution was less than 30–50%; when the dilution was greater, the increase in food intake did not compensate for the dilution. Kennedy (1950) confirmed this observation for normal rats given kaolin-diluted diets, but found that rats with hypothalamic lesions ate less of the diluted diets. This behaviour he put down to an increased discrimination of the hyperphagic and the obese rats against diets made unpalatable with kaolin.

The purpose of the study reported here was to determine whether rats with lesions of the ventromedial nuclei, given a choice between two diets of differing caloric density, are able (1) to discriminate between the diets, and (2) to choose the proportion of the diets in relation to their calorie needs.

EXPERIMENTAL

Diets

The experiment consisted of presenting each rat with a choice between two diets, one undiluted (diet A) and one diluted (diet B or diet C). The composition of the basic diet A is shown in Table 1. Diet B was made by adding 15 g cellulose powder

(Whatman, Grade B) to 100 g basic diet; diet C was made by adding 30 g cellulose powder to 100 g basic diet.

The energy values of the diets were: diet A 4·6, diet B 4·0 and diet C 3·5 kcal/g.

Animals

Male hooded rats, aged 53–59 days at the beginning of the experiment, were allotted at random to two groups; those in group 1 were offered a choice between diets A and B and those in group 2 a choice between diets A and C. Both food and water were offered *ad lib.*

Each rat was housed separately in a special cage. The cage was designed so that it was symmetrical in every detail about a mid-line dividing it from back to front. Thus, the two food pots were presented to the rat with no extraneous clues from the cage that might provide differential stimuli. The pots were fixed in holders in the front of the cage, and separated by a metal partition, higher than the top of the pots. There was also a partition under the floor grid to ensure that spillage from each pot could be weighed separately. The water bottle was located at the back of the cage.

Brain lesions

The brain lesions were made under pentobarbitone anaesthesia with a Stoelting Stellar Stereotaxic instrument. The animal was held in the instrument, its skull exposed and a hole made on each side of the sagittal fissure with a dental trephine. The lesions were made with the co-ordinates 1·5 mm posterior to the bregma, 0·5 mm lateral to the mid-line and 9·3 mm from the surface of the dura. An insulated unipolar electrode, diameter 0·25 mm, with 0·25 mm of the tip exposed, was used to pass a direct current of 2 mA for 15 sec. After the electrolytic lesions had been made, Polybactrin (bacitracin-neomycin-polymyxin; Calmic Ltd) was sprayed over the skull, and the skin sutured. An intramuscular injection of penicillin was given to reduce the risk of infection. Histological examinations of the brains of the operated animals were made at the end of the experiment to determine the exact site of the lesions. Control rats were 'sham-operated' by anaesthetizing them and exposing the skull, stopping short before the drilling of the skull. Like the operated rats, the sham-operated ones were treated with Polybactrin and penicillin.

Procedure

For a period of 50 days before operation each animal was presented daily with 50g diet A and 40 g diet B or C; these amounts had approximately equal volumes. Different sets of food pots were used each day, and the positions of the food were changed each day according to a predetermined random order. The intake of each diet was measured daily, and the rats were weighed three times weekly.

The rats were then operated, or sham-operated, and the same feeding procedure was continued. Water consumption and food consumption were both measured, in order to determine whether besides hyperphagia polydipsia had been produced by the operation. As usual in this type of work, more animals were used than were later found to be suitable for experiment. We began with thirty rats, so as to obtain eight hyperphagic animals and eight control, sham-operated animals. The experimental and control rats were each divided into two groups with four rats in each, containing the experimental (operated) animals (groups 1*x* and 2*x*) and the control (sham-operated) animals (groups 1*c* and 2*c*). Groups 1*x* and 1*c* were choosing between undiluted diet A and diluted diet B; groups 2*x* and 2*c* were choosing between undiluted diet A and diluted diet C.

Table 1. *Composition of the purified diet*

100 parts diet contained:

Sucrose	60	Arachis oil	15
Low-vitamin casein	20	Salts	5

B vitamins were added so as to give, in 100 g diet:

Choline	100 mg	Pyridoxine	800 μg
Inositol	22 mg	Thiamine hydrochloride	500 μg
Nicotinic acid	10 mg	Folic acid	100 μg
Calcium-D-pantothenate	10 mg	Biotin	20 μg
p-aminobenzoic acid	7·5 mg	Cyanocobalamin	0·01 μg
Riboflavin	3 mg		

Supplements given to each rat once weekly were 1 mg α-tocopherol and, on a different day, 120 i.u. vitamin A, 20 i.u. vitamin D, and 500 μg menaphthone.

RESULTS

A one-way analysis of variance (Kruskal & Wallis, 1952) showed no significant differences in body-weight between the four groups before the operations. After operation the four rats in each of the groups 1*x* and 2*x* showed hyperphagia, which we defined as a weight gain of at least 25 g during the week after the operation. The water intake of these rats was about 1 g for each g food eaten and somewhat more when the diluted diets were eaten. The differences between various groups, described below, were tested for significance by the method of Mann & Whitney (1947).

Weight gain

The gain in weight of the sham-operated animals did not show appreciable change after the operation; the gain in weight of the animals with hypothalamic lesions increased considerably immediately after the operation and continued at this same rate for several weeks (Figs. 1 and 2). The weight of each of the obese rats eventually reached a plateau, which we defined as a weight that did not increase over a period of 7 days. The increase in weight of the obese rats, from the day of operation to the day when maximum obesity was reached, ranged from 162 to 342 g in group 1*x* and from 114 to 291 g in group 2*x*. The increase in weight of the control rats, from the day on which they were sham-operated to the day on which the experiment was ended, ranged from 61 to 69 g in group 1*c* and from 66 to 88 g in group 2*c*. The differences both in weight and in weight increases between groups 1*x* and 1*c* and between groups 2*x* and 2*c* were significant ($P = 0·02$).

Food choice

The behaviour of the rats in choosing their diet was highly consistent. The choices of the operated rats, in both group 1*x* and 2*x*, were closely similar during the various phases, as were the choices of the control rats in groups 1*c* and 2*c*. Typical records for two rats, one from group 2*x* and the other from group 2*c*, are shown in Fig. 3. Before operation both rats chose to eat some of each diet. However, after damage to

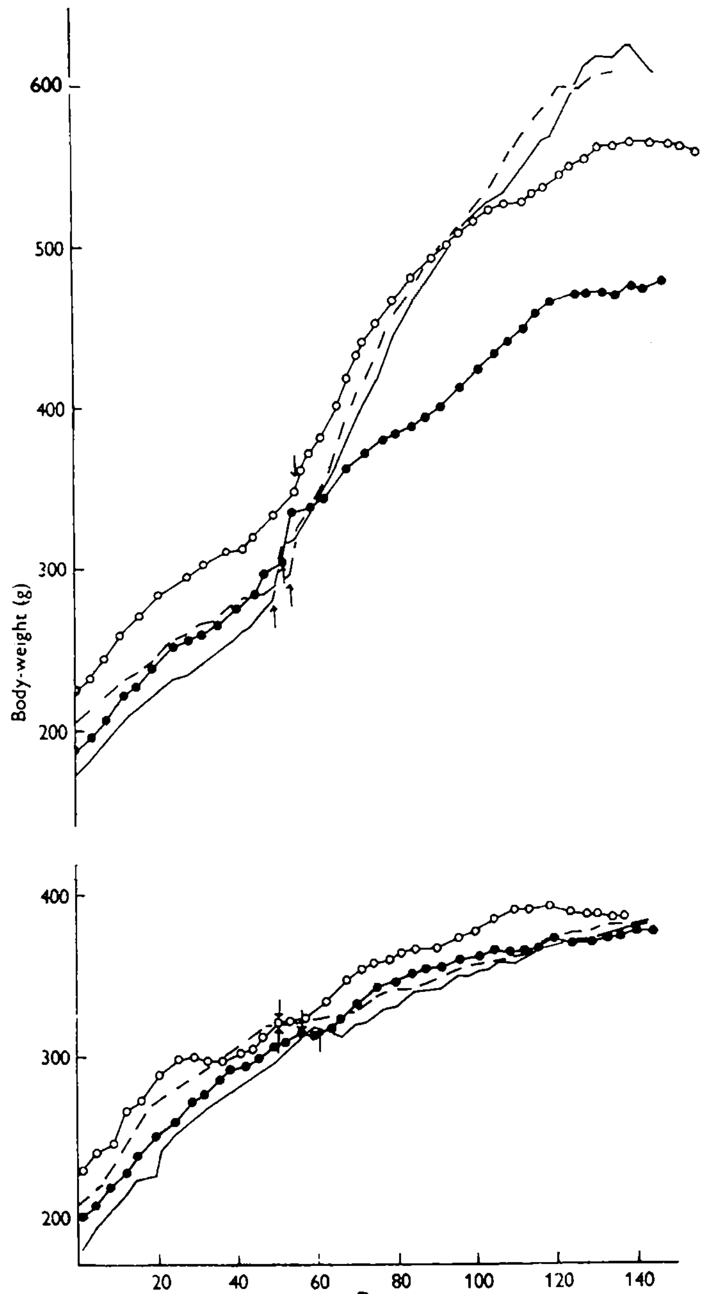

Fig. 1. Body-weight curves of four rats with ventromedial lesions (upper) and of four sham-operated rats (lower). Rats offered choice between undiluted diet A and diluted diet B (100 g diet A + 15 g cellulose powder). Arrows show time of operation or sham operation.

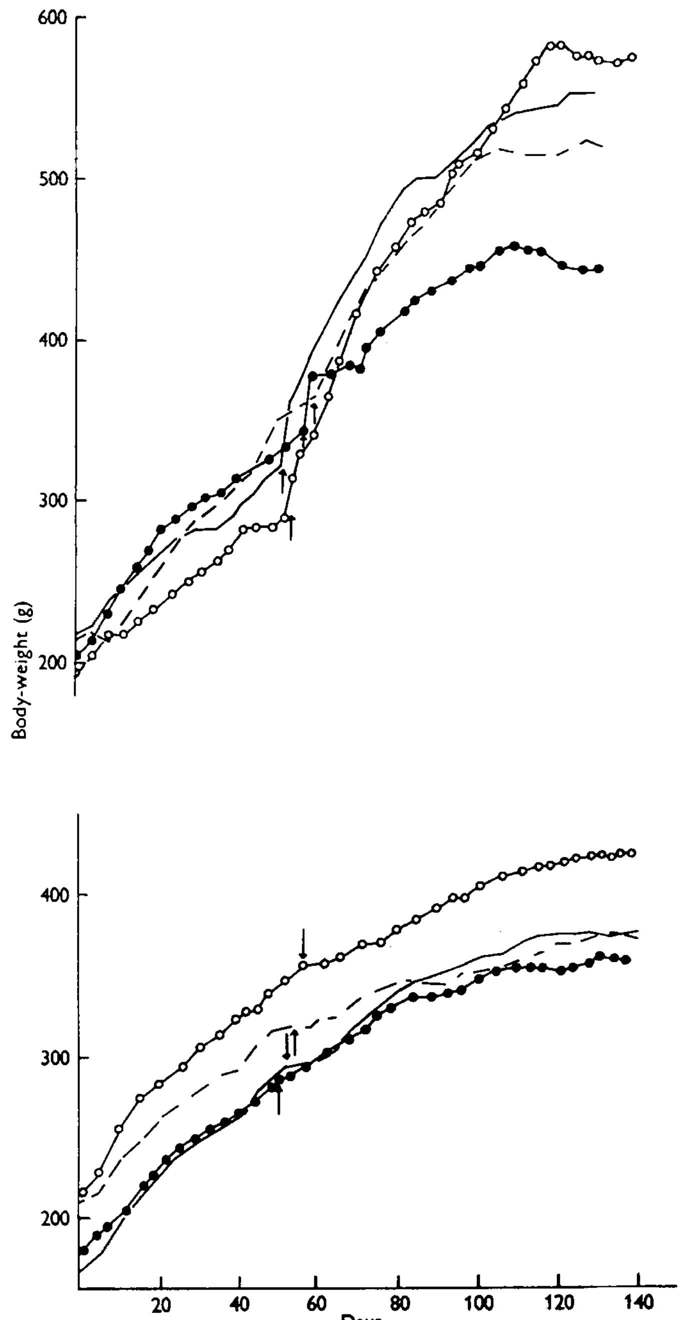

Fig. 2. Body-weight curves of four rats with ventromedial lesions (upper) and of four sham-operated rats (lower). Rats offered choice between undiluted diet A and diluted diet C (100 g diet A + 30 g cellulose powder). Arrows show time of operation or sham operation.

the ventromedial nuclei, there was a considerable change in food preference. From then on, the operated rat ate the concentrated diet almost exclusively, until it reached maximum obesity. It then exhibited a second change in food preference and ate mostly the diluted diet. No such changes were observed in the control, sham-operated rat, which throughout ate similar amounts of both diets.

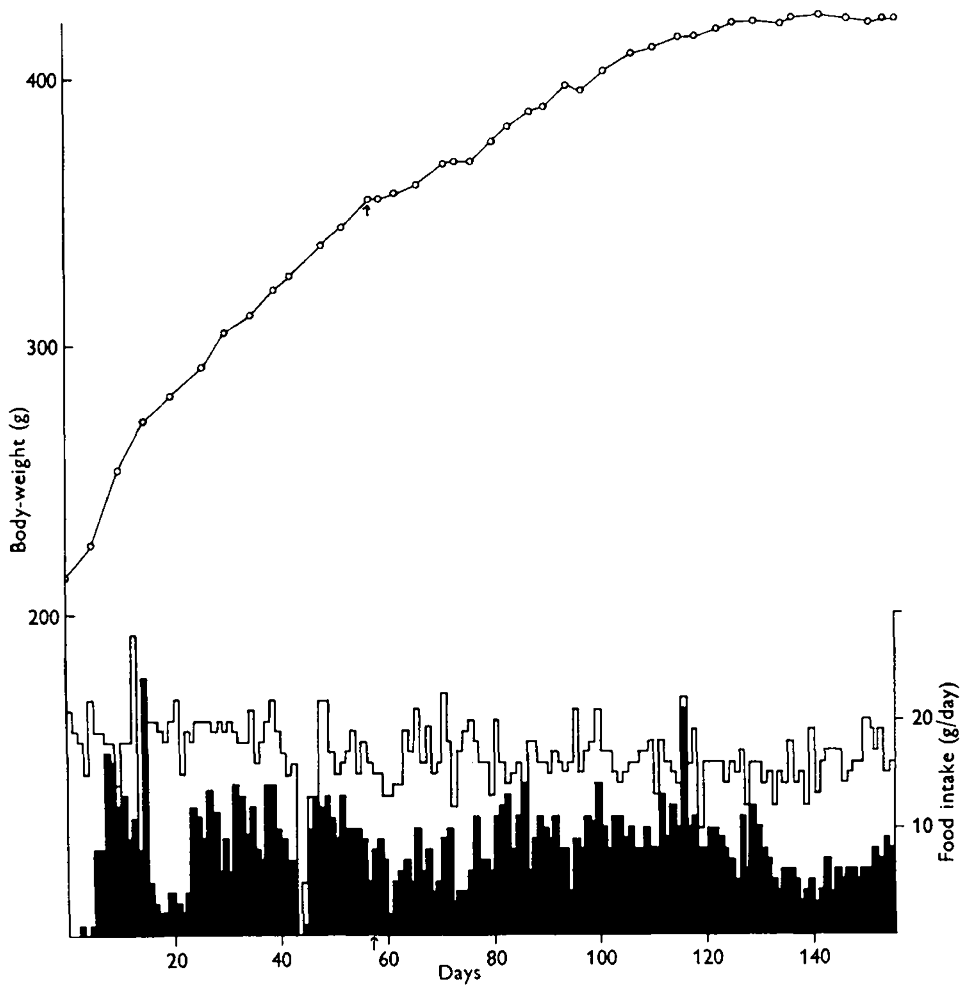

Fig. 3. Body-weight and food intake of a rat with ventromedial lesions (right) and of a sham-operated rat (left) offered choice between diet A and diet C (100 g diet A + 30 g cellulose powder). Black areas, intake of diet A; white areas, intake of diet C. Arrows show time of operation or sham operation.

We have compared the food intakes of the operated and sham-operated rats during three stages of growth, (1) period of normal growth, before operation, (2) period after operation, and (3) period of maximum obesity. Fig. 4 shows the food intake during the period of normal growth. The proportion of diet A eaten, as a percentage of total food intake, during the first 21 days was not significantly different between groups 1*x* and 1*c*, or between groups 2*x* and 2*c*.

Fig. 5 shows the food intake during the 21 days after the operation. Here, there was a great increase in total food intake and a strong preference for the concentrated diet in the rats with hypothalamic lesions, groups 1*x* and 2*x*. The differences from the

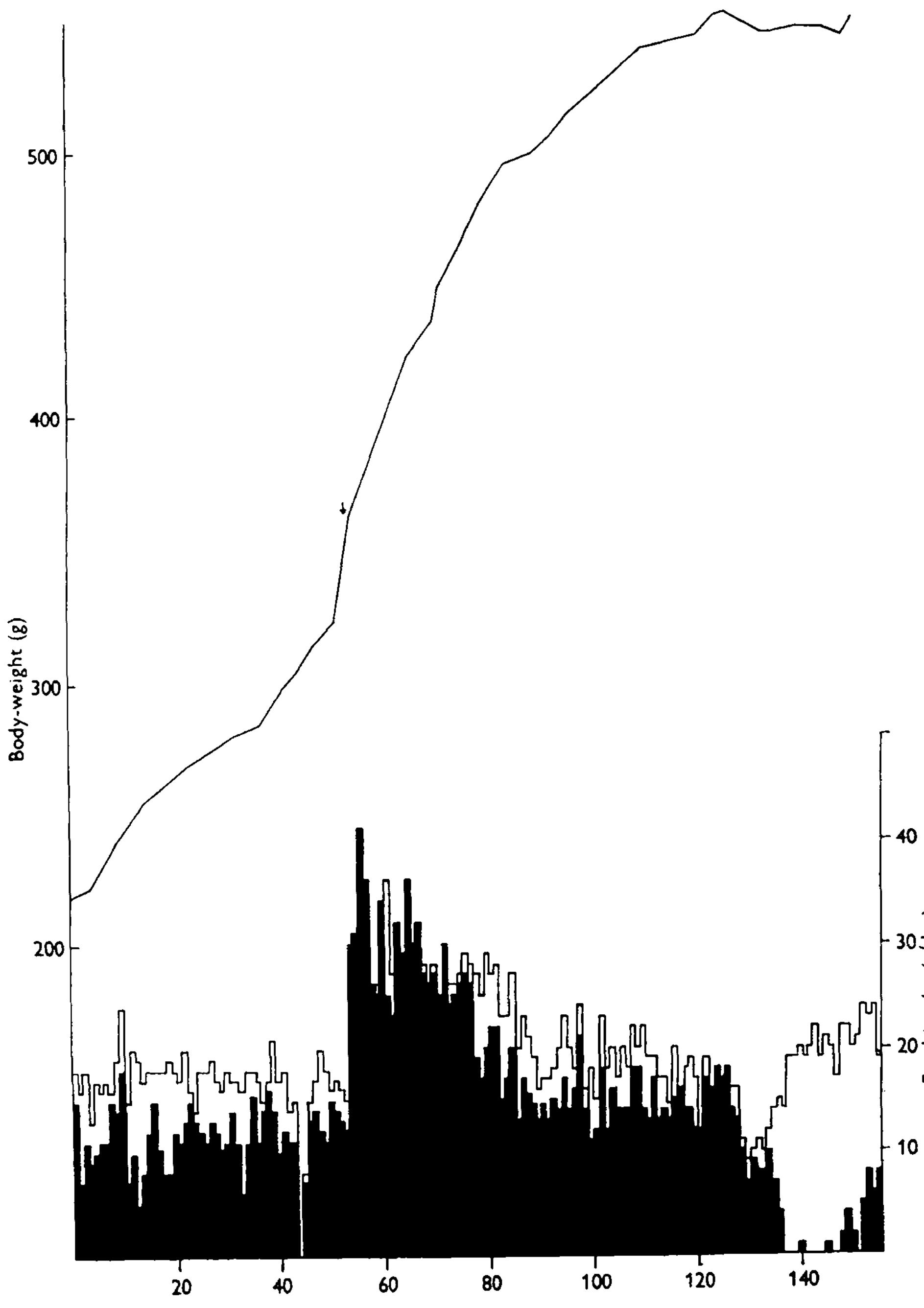

Fig. 3 (*continued*). For legend see facing page.

controls, again expressed as a percentage of total diet eaten, were significant (groups 1*x* and 1*c*, $P = 0.02$; groups 2*x* and 2*c*, $P = 0.05$).

Fig. 6 shows intake during the 21 days after maximum obesity had been reached by the hyperphagic rats. The food consumptions of the obese rats in groups 1*x* and 2*x* were then about the same as those of the rats in the control groups 1*c* and 2*c*. In this

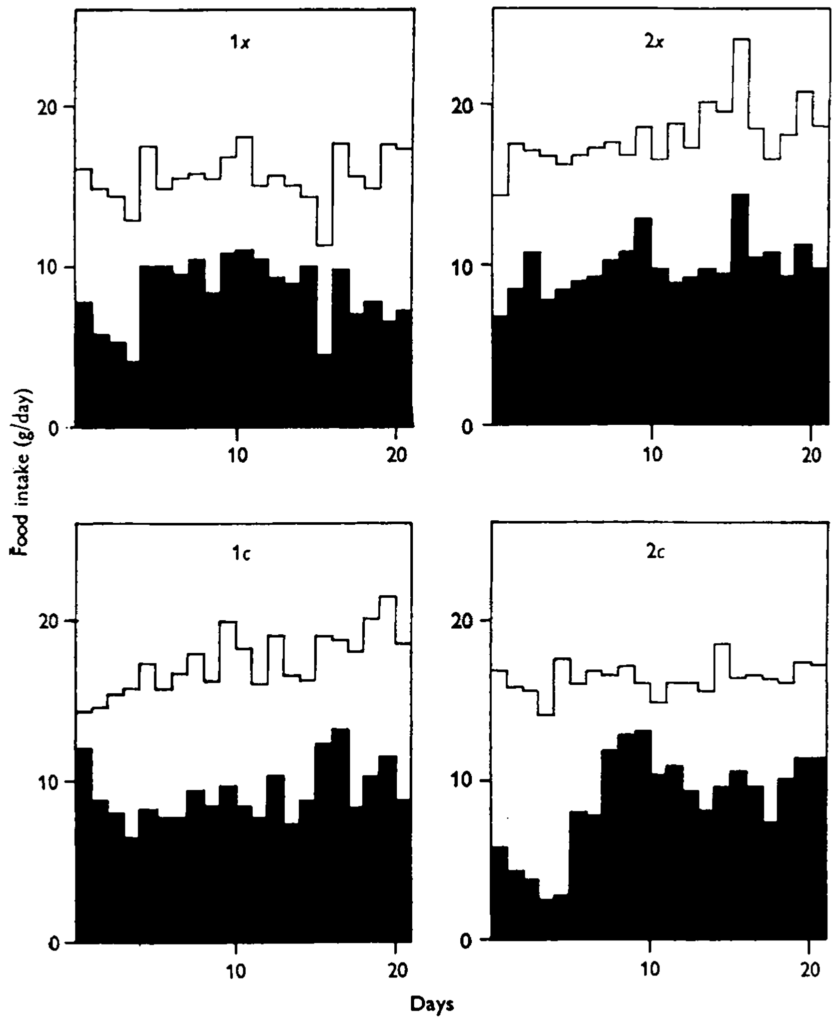

Fig. 4. Mean food intake of four rats during 21 days of pre-operative period of maximal normal growth. 1*x* and 1*c*, rats choosing between diet A (black areas) and diet B (white areas); 2*x* and 2*c*, rats choosing between diet A (black areas) and diet C (white areas); 1*x* and 2*x*, experimental rats, later operated; 1*c* and 2*c*, control rats, later sham-operated.

phase, the food preference of the obese rats had changed again, more diluted diet than concentrated diet being consumed. As in the first, pre-operative phase, there was again no significant difference in the proportion of diet A eaten between either of the obese groups and its respective control group.

The control groups 1*c* and 2*c* gradually increased their consumption of diluted diets throughout the experiment. In contrast, as we have seen, the operated groups suddenly changed their preference to the concentrated diets during the hyperphagic phase and then suddenly back to the diluted diets during the phase of maximum obesity. This

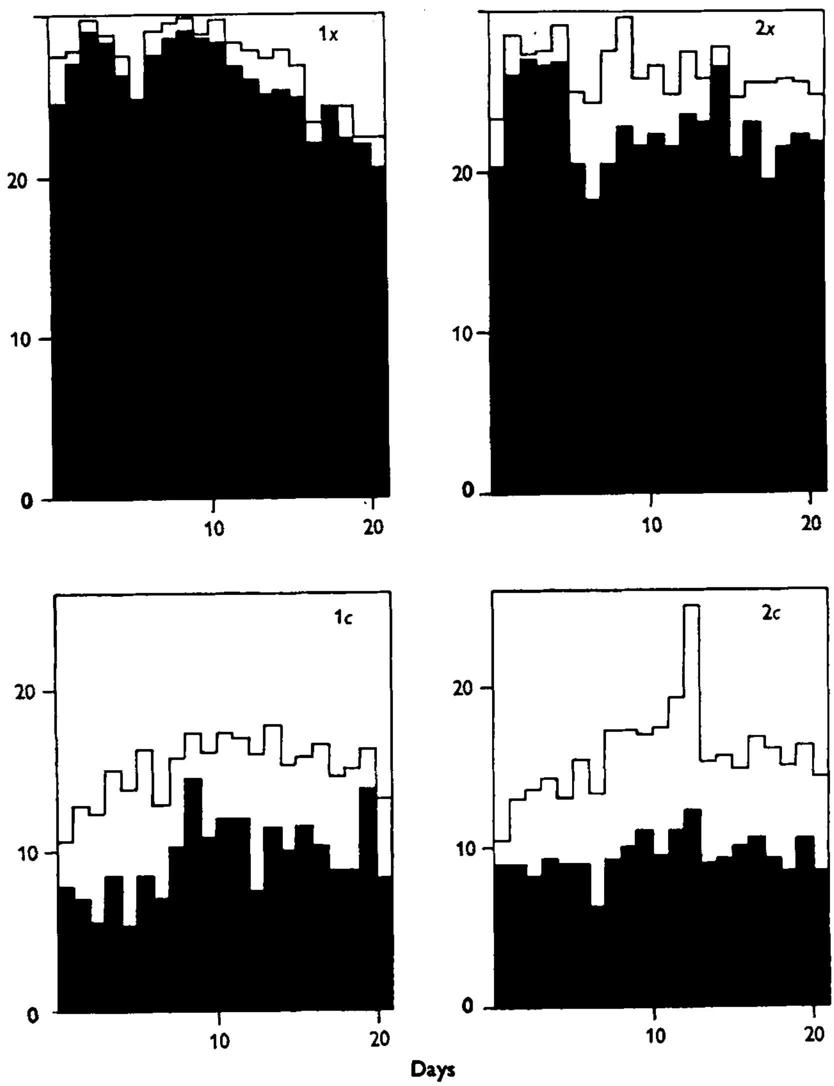

Fig. 5. Mean food intake of four rats during 21 days immediate postoperative period. 1*x* and 1*c*, rats choosing between diet A (black areas) and diet B (white areas); 2*x* and 2*c*, rats choosing between diet A (black areas) and diet C (white areas); 1*x* and 2*x*, operated rats, 1*c* and 2*c*, sham-operated rats.

latter shift by groups 1*x* and 2*x* was significantly greater than the much smaller and gradual change in the same direction by the control groups 1*c* and 2*c* ($P = 0{\cdot}05$ in each instance). There was no significant difference in the proportions of diet A consumed in the pre-operative phase and the obese phase in either the operated or the control groups.

Brain lesions

Histological examination of the brains of the operated animals at the end of the experiment confirmed that the eight hyperphagic animals described above had suffered destruction of the ventromedial nuclei (Pl. 1). Two of the operated animals,

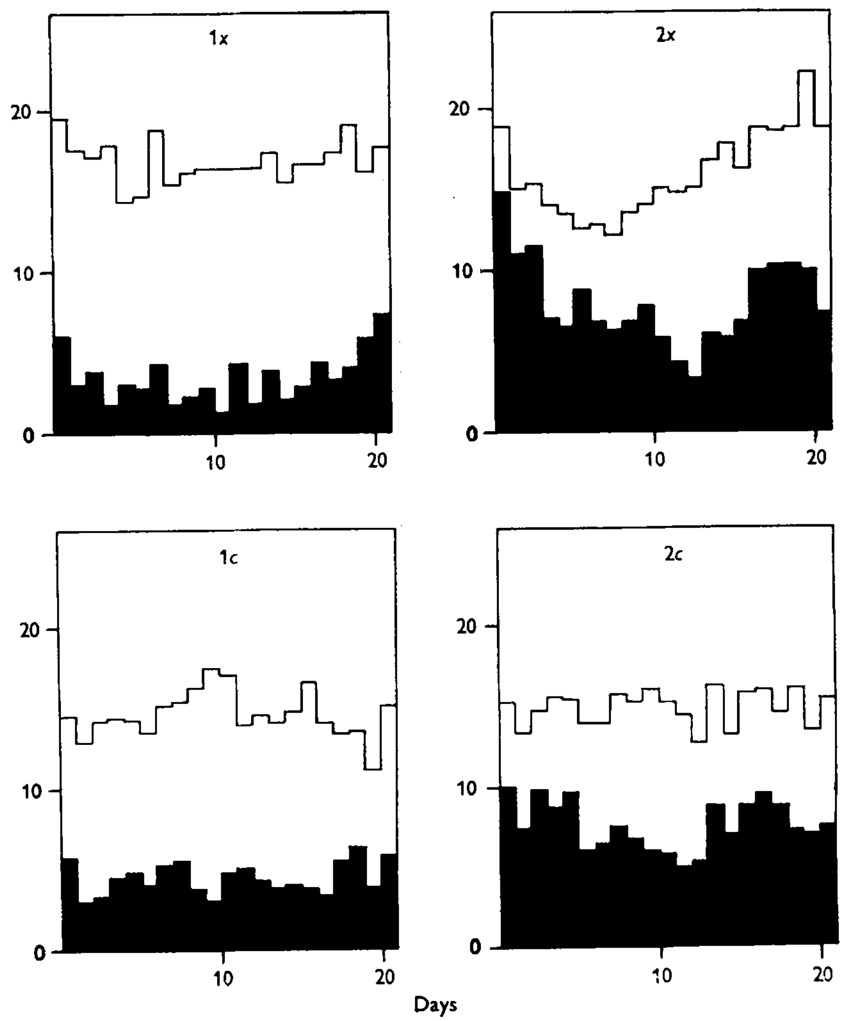

Fig. 6. Mean food intake of four rats during 21 days after the operated rats had attained maximum obesity in the postoperative period. 1*x* and 1*c*, rats choosing between diet A (black areas) and diet B (white areas); 2*x* and 2*c*, rats choosing between diet A (black areas) and diet C (white areas); 1*x* and 2*x*, operated rats; 1*c* and 2*c*, sham-operated rats.

which had shown no hyperphagia, obesity or changes in food preference and whose behaviour has not been included in this record, had lesions in the posterior hypothalamic region but not in the ventromedial nuclei.

DISCUSSION

The exhibition of a preference towards diet A by the operated rats of group 1*x* suggested that the animals were able to discriminate between calorie concentrations of 4·6 and 4·0 kcal/g; the preference seemed as evident as that shown by rats of group 2*x*, with the greater difference in calorie concentration of 4·6 and 3·5 kcal/g. The food preference was seen even when the rats must still have been under the influence of the operation, since it was evident during the first 24 h after the lesions had been made. That the lesions of the ventromedial nuclei were responsible for the change in food choice was shown by the absence of the new preference both in the sham-operated rats of groups 1*c* and 2*c* and in the two operated rats in which the lesions had been misplaced.

Kennedy (1950) has shown that, compared with normal rats, animals offered a diet containing 50% kaolin ate less during the hyperphagic phase and still less during the obese phase. He concluded that rats with lesions of the ventromedial nuclei were more discriminating against unpalatable diets than normal rats. Similar effects of unpalatability on hyperphagic and obese rats with hypothalamic lesions were shown by Miller, Bailey & Stevenson (1950) who added quinine to the diets of the rats.

From Kennedy's experiments, one might then have predicted that our hyperphagic rats, given a choice between diluted and undiluted diets, would have eaten more of the undiluted diet as being more palatable. Although we used cellulose rather than kaolin, and although we used lower dilutions than 50%, we did find the expected preference for the undiluted diet. But if this preference had been due to the unpalatability of the diluted diets, then we should have predicted that the rats during the obese phase would have eaten even less of the diluted diets. It did not happen, for they ate much more of the diluted diets. Thus, anyhow at the concentrations used in our experiment, additions of cellulose do not produce diets unpalatable to rats with hypothalamic lesions, as does the addition of 50% kaolin.

With our diets, the preferences of the hypothalamic rats reflected their calorie needs. During the phase of hyperphagia, when the animal was putting on most weight and had a large calorie need, it showed a strong preference for the concentrated diet. On the other hand, during the phase of reduced food intake, when the animal was only maintaining its obesity and had a small calorie need, the preference was for the diluted diet, which provided fewer calories for a given weight of diet. Though the rat may thus discriminate between the concentrated and the diluted diets by using taste, texture, smell and perhaps other sensory clues, its preference is affected by its calorie needs. More particularly, change in calorie need seems to determine immediate change in preference.

The increased food intake of the animal with lesions of the ventromedial nuclei poses two possibilities, which we can formulate as follows. Does the animal eat more because it has an increased desire to chew and swallow food, or does it do so in order to satisfy some biochemical expression of the need for calories? The results of our experiments are compatible with the second suggestion rather than the first, that it eats for calories rather than to satisfy an increased desire for mechanical eating activity.

On the other hand, the aphagia produced by lesions from the lateral hypothalamus is probably due to an inhibition of eating activity and not to a decreased demand for calories, according to Baillie & Morrison (1963).

SUMMARY

1. Thirty rats aged about 8 weeks were offered the choice between an undiluted purified diet and either of two diluted diets, made by adding 15 or 30 g cellulose to 100 g of the undiluted diet.

2. After 50 days, some rats were subjected to operation that produced lesions of the ventromedial nuclei of the hypothalamus, and other rats were subjected to sham-operation.

3. Four groups of four rats each were constructed, containing the experimental (operated) rats or the control (sham-operated) rats and continuing to choose between the undiluted and one of the two diluted diets.

4. The operated rats, in which lesions of the ventromedial nuclei were later confirmed histologically, developed typical hyperphagia and gained weight rapidly until they reached a fairly steady weight and their food intake fell to that of the control rats.

5. Before operation all rats ate about equal quantities of the undiluted and diluted diets. After operation the control rats continued to choose in a similar way, except that they gradually ate a slightly higher proportion of the diluted diet as the experiment continued.

6. The experimental rats ate a significantly higher proportion of the undiluted diet during the hyperphagic phase, from the time they recovered from the anaesthetic until the time they reached maximum obesity. During the obese phase, they ate more of the diluted diet, in the same proportion as the control rats.

7. It is concluded that, under the conditions of this experiment, rats with lesions of the ventromedial nuclei eat for calories, choosing the proportions of a diluted and an undiluted diet in relation to their calorie needs. They are able to distinguish between the different caloric densities of the diets as soon as their calorie needs change.

We take this opportunity of recording our gratitude to Genatosan Ltd, Loughborough and to the Energen Foods Company, Ashford, Kent for their generous support of the research programme of this laboratory.

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EXPLANATION OF PLATE

Transverse section of brain of successfully operated rat. Lesions exactly in area of ventromedial nuclei; third ventricle symmetrically enlarged from normal slit-shape, because of scarring produced by lesions.

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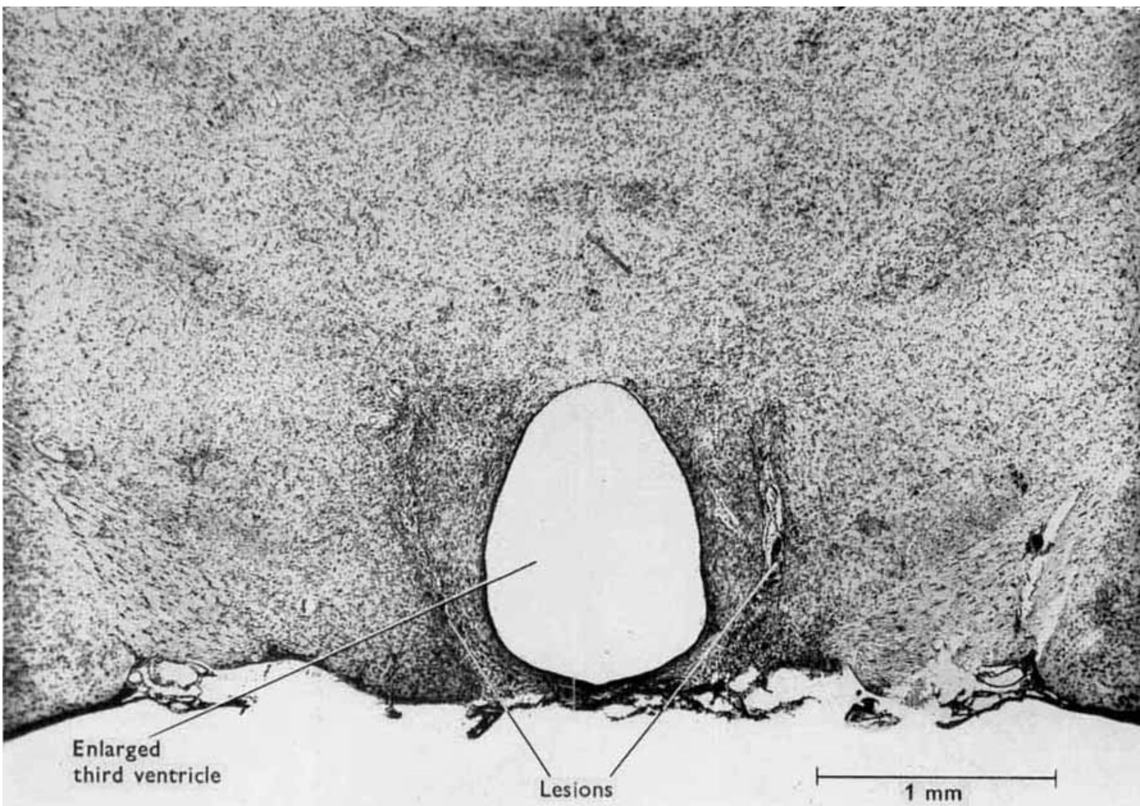

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