Adipsia and Aphagia in Rats After Lateral Subthalamic Lesions

SEORAS D. MORRISON AND JEAN MAYER

From the Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts

ABSTRACT


Localization of lesions in the lateral subthalamic area of rats which produce a high success rate of aphagia is described. Complete adipsia accompanied this aphagia in all cases. The aphagia and the adipsia are separate responses to the lesion. The pattern of material exchange cannot be reproduced by deprivation of sham-operated rats of either food or water. Rats fully sham operated in this area show hypophagia and hypodipsia for 1 or 2 days after operation. Daily intubation of water into operated animals appears to facilitate the escape of animals from the inhibition of eating and drinking. Escaped animals showed a pronounced polydipsia after escape, but this was of a variable degree of permanence and did not always appear consistent in other respects with diabetes insipidus.

It has been shown by Anand and Brobeck (1, 2) for cats and rats that electrolytic lesions in the lateral hypothalamus induce aphagia which persists until death of the animal from fasting. These authors made no mention of changes in water intake, but Teitelbaum and Stellar (3) have recorded that adipsia also occurs after these lesions. Montemurro and Stevenson (4) made lesions in this area and observed varying occurrence and association of aphagia and adipsia. It seemed to us important to examine whether the observed aphagia was merely a physiological consequence of this adipsia or whether both phenomena were directly the result of the lesions, although not necessarily independent.

METHODS

Adult female albino rats of the Sprague-Dawly strain were used, of 230-270 gm initial body weight. Symmetrical, bilateral lesions were made using the stereotaxic instrument described by Krieg (5). Direct current at 40 v and 2 ma was passed for 15 seconds with the electrode point at the appropriate place. Initially lesions were made in the same coronal plane as those producing hyperphagia (that of the median eminence) and 1 mm laterally to that site (i.e. 3 mm apart) and $\frac{1}{2}$-1 mm from the floor of the brain. These lesions failed to produce aphagia in any animal as measured by continuous loss of body weight. On the basis of Anand and Brobeck’s (2) description and Krieg’s (5) diagrams of the rat hypothalamus, and the diagram by Mayer et al. (6) of the corresponding position in the mouse, the coordinates were altered. The new coordinates again lie in the same coronal plane as for hyperphagic lesions (i.e. 6 mm caudal to the ear bars, 1-2 mm caudal to the bregma), 2 mm lateral to the mid-line (sagittal suture) and 2 mm dorsal to the floor of the brain (the sphenoid). The electrode is inserted vertically until it touches the sphenoid and is then withdrawn 2 mm. The centers of the lesions thus lie in the same coronal and sagittal planes as those described by Anand and Brobeck (2), but 1 mm dorsal to them. Placing the lesions in this way has given 20 successes out of 21 trials. Of these 20, a total of 8 animals has, subsequently, ‘escaped.’ By this term, here
and elsewhere in this paper, we mean animals which have, after a period of complete aphagia and adipsia, spontaneously resumed drinking and eating and, eventually, have regained their initial body weight.

In the initial experiments (eight animals) only body weight was recorded. In a group of four operated animals with three unoperated controls body weight and daily water intake were recorded. In the final experiment with 30 animals body weight, water intake and food intake were recorded daily. Water intake was recorded as volume, using graduated water tubes. Food was a stock diet (Purina laboratory chow) ground to powder and fed in tared "scatter-proof" cups.

The final and definitive experiment was designed with five groups of six rats each. Body weight was recorded for 4 days before operation, and food and water intake for 3 days. One group was operated with bilateral lesions. One group was also operated with bilateral lesions, and after the second postoperative day these animals were intubated daily with 10 ml of water. Three groups were sham operated to the extent of inserting the electrode to the floor of the brain and then withdrawing, that is, an identical procedure in every way except that no current was passed. Of the sham-operated groups one was a simple control allowed food and water ad libitum. One was allowed water ad libitum but was given no food. One was allowed food ad libitum but no water. Half of each of the restricted control groups (three animals) had free access to food and water restored after 6 days postoperatively, and the other three animals had them restored after 7 days. A further control group of two animals was sham operated to the extent of inserting the electrode 6 mm below the dura mater; this amounts to the needle point stopping about 4 mm above the sphenoid or 2 mm above the center of the lesion in the operated animals.

Brains of representative animals (10) were fixed in neutral 10% formalin, and serial sections were cut at 8 μ and stained with hematoxylin and eosin to allow definition of localization of the lesions.

RESULTS

The positions of the lesions, established from the serial sections of 10 brains, were as follows. The lesions were large, approximately 1 mm ventro-dorsally, 0.5 mm laterally and 1.5 mm rostro-caudally. They extended from just rostral to the posterior hypothalamic nuclei to just caudal to the supra-optic nuclei; and from just dorsal to the fornices to the floor of the brain. The lesions were lateral to the fornices and in some cases touched the inner boundary of the internal capsule (figs. 1 and 2). The supra-optic nuclei, ventro-medial hypothalamic nuclei and the fornices were undamaged.

The body weights of the operated groups fell precipitously in the first 2 days postoperatively to 82% of the initial weight (fig. 3). Thereafter, the body weights of the main experimental groups declined at a lower but still rapid rate. The mean body weight at death of the rats of the first group was 55% of the initial weight. After the 2nd day the body weights of the intubated animals (i.e. after the beginning of intubation) declined more slowly than those of the main group reaching 71% of initial on the 6th day compared with

FIG. 1. Coronal section of rat hypothalamus through median eminence and centers of lateral lesions. Magnification X 39.
66% for the unintubated animals. The body weights of all the control groups dropped in a similar way during the 1st postoperative day. The unrestricted control group showed a further slight fall in weight on the 2nd day and then recovered slowly, regaining their initial weight by about the 10th postoperative day. The sham-operated group deprived of water lost weight throughout at a rate almost identical to that of the fully operated group, until access to water was restored at the 6th and 7th days (fig. 4). The sham-operated group deprived of food lost weight less rapidly, reaching 73% of initial body weight on the 6th day (fig. 4). The weight loss of this food-deprived group was very slightly less than that of the operated intubated group.

The water intake of all groups fell to or near zero for the first postoperative day (fig. 4). The voluntary water intake for the two operated groups fell to zero on the 1st day postoperatively and remained at a mean intake of 0.7 ml/day. Even this small amount is largely accountable to evaporation and spillage, as the operated animals frequently attacked their water tubes when these were being put in the cage, even though they did not drink. (This average apparent intake excludes the intakes of escapees after the beginning of escape; this is dealt with later.)

The fall in water intake of the unrestricted control group was to 10% of the preperiod mean and the intake did not recover immediately to the normal level, being only 50% of normal on the 2nd day. The control group deprived of food showed a fall in water intake to zero on the 1st day with a recovery to 25% of normal on the 2nd and 3rd days. Thereafter it declined irregularly until the 7th day (fig. 4d). When food was restored to these animals the water intake rose abruptly to about twice the preperiod level and declined to normal in the next 1 or 2 days. A similar abrupt rise in water intake occurred on restoration of water to the water deprived group.

The food intake of all groups fell to near zero on the 1st postoperative day. The food intake of both operated groups fell to zero on the first postoperative day and remained at a mean apparent intake of 0.75 gm/day. (Again this average intake excludes the intakes of escapees after escape.)

The food intake of the unrestricted control group fell to 20% of normal on the first day and then recovered slowly to reach normal intake after 6 or 7 days. The food intake of the group deprived of water fell to 25% of preperiod intake in the 1st day and thereafter declined slowly (fig. 4e). Restoration of food on the 6th day to the food-deprived group produced an immediate recovery of food intake to 130% of preperiod levels; restoration on the 7th day produced an immediate rise only to 90% of preperiod. Restoration of water to the water-deprived group produced a recovery of food intake to about 85% of normal on the 1st day and exceeded preperiod levels on the 2nd day of restoration (fig. 4e).

The responses of the intubated group are confused by the occurrence of escapes throughout the postoperative period. Of the six animals operated and unintubated none escaped. Of the six intubated rats, one died at 7 days and one at 9 days, possibly as a result of pulmonary inflammation or infection through a small amount of water accidentally passing into the trachea, but both times of death fall within the range of survival time shown by the unintubated group. The re-
remaining four animals escaped at 4, 9, 10 and 13 days by the criterion of reversal of body weight change, and at 3, 8, 10 and 10 days by the criterion of appreciable voluntary water intake. Appreciable food intake occurred by 3, 8, 10 and 10 days, respectively. There was some indication that recovery of water intake preceded recovery of food intake. Even assuming that the intubated animals which died would not have escaped, the relation of four escapees from one group and none from the other is statistically significant (P < 0.02).

The escapees showed a rise to normal fluid intake over 1-4 days (fig. 5) from the first appearance of voluntary water intake. They then showed a further rise in water intake to about 150–200% of normal. Restriction to normal intakes (ca. 30 ml/day) for 2 days produced no appreciable weight loss in three animals, and on restoration of water ad libitum the intakes of these three remained only slightly above the normal level (fig. 5a). The body weight did not fall on water restriction but it rose very markedly when the restriction was removed although the water intake stayed only slightly above normal. The remaining escapee lost weight markedly during water restriction and the subsequent ad libitum intake returned to the raised level (fig. 5b).

The mean body weight at death of the operated animals was 137 gm or 55% of the initial weight. The mean time of death postoperatively was 10 days. The two groups of restricted sham-operated animals both completely recovered their body weights and food and water intakes after removal of the restrictions. When the restriction on water intake was removed after 6 days, the rats immediately started to drink large quantities of water (fig. 4e) and the recovery was rapid. When the restriction was removed after 7 days the rats showed less inclination to drink, and two animals had to be lifted and placed in front of the water tubes. These apparent very high intakes may be excessive as a considerable degree of 'slopping' occurred during the initial period of drinking. It seems likely that 7 days of total water deprivation is approaching the limit of voluntary reversibility of dehydration in the rat. The water intake on restoration of unrestricted food to the food deprived group was also large for the first 2 days but there was no difference in this response after 6 or 7 days of deprivation.

All the fully sham-operated animals showed pronounced hypophagia and hypodipsia for the 1st postoperative day, with only slight recovery on the 2nd day (fig. 4), and also lost weight at the same rate as the operated animals over this period. Thereafter they regained normal body weights and food and water intakes over a period of 7-10 days. The partially sham-operated controls, however, dropped to only 95.5% of initial body weight during the 1st postoperative day, compared with 90.5% for the fully sham-operated ani-
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ESCAPEE—PATTERN OF ESCAPE

DAYS

INTUBATED WATER

VOLUNTARY WATER

BODY WEIGHT

FIG. 5. Pattern of release of inhibition of eating and drinking of two rats which showed (a) temporary polydipsia and (b) persistent polydipsia.

animals, and had recovered normal weight by the 3rd day (fig. 3). Water intakes of these animals dropped to about 45% of normal in the 1st postoperative day but had recovered to normal on the 2nd day. Adequate control food intakes are not available for these animals but their intakes on the 1st day were about 30% of normal with complete recovery by the 3rd day. This was a similar response to that of rats sham operated in the medial region (i.e. for lesions of the ventro-medial nuclei to produce hyperphagia).

One of the fully sham-operated rats remained aphagic and adipsic and followed an exactly parallel course to the operated animals. (This has happened with one other fully sham-operated animal in another experiment.) It was killed for histological examination 11 days postoperatively at a body weight of 148 gm (57% of initial). This animal maintained a slight water intake throughout (mean 4.7 ml/day).

DISCUSSION

The fact that both adipsia and aphagia occur immediately as a result of these lateral lesions gives rise to the possibility that the lesions may produce only one effect, the other being a peripheral physiological consequence. It is apparent from the experiments described here, however, that neither complete food deprivation nor complete water deprivation in sham-operated animals is capable of simulating the pattern of exchange of materials found in the operated animals. Complete deprivation of water did produce a fall in body weight almost identical to that produced by the lesions (fig. 3) but the food intake in the sham-operated animals deprived of water remained well above zero, and significantly above that of the fully operated animals (P < 0.01), for 5 days after the operation. In contrast the food intake of the intubated rats did not differ significantly from that of the operated unintubated animals. Complete deprivation of food produced a much slower rate of loss of body weight than in the operated animals, and also the water intake of the food-deprived group was, for the first 5 days, significantly above that of the operated animals (P < 0.001). It can be concluded, therefore, that the lesions produce both the effects of adipsia and aphagia directly. From the identity of body weight loss in the operated and in the water-deprived animals it might be suggested that the adipsia is the dominant effect. The slight indication found here and in the work of Teitelbaum and Stellar (3) that escaped animals tend to drink before they eat would support this contention. On the other hand, the turnover of water is much greater than that of energetic materials in terms of weight, and the identity of body weight change in the two groups is also simply explicable on the basis of the relatively large mass of daily obligatory water loss. The necessity of restoring this large mass of water could also be held simply to explain the slightly earlier recovery of water intake and great excess of initial water intake over initial food intake in the first stage of recovery of restricted animals.

In none of our experiments has adipsia been produced independently of aphagia. Such an effect would have appeared either as an appearance of food intake in the intubated animals without voluntary water intake, or, in the unintubated animals, as a decline in voluntary food intake similar to that of the water-restricted group and not an abrupt cessation of food intake. Thus, even if there is a dominance of adipsia over aphagia and not simply a dominance of water loss over energetic material loss in terms of mass, it would appear that the two responses are closely connected. Either the same structures control both responses, or the structures controlling each are so intimately associated anatomically as to be not easily susceptible of discrimination by the lesioning method. Greer (7) has produced 'pure drinking' in one rat by electrical stimu-
lation of the hypothalamus, and Montemurro and Stevenson (4) have reported results of one rat which became adipsic but which ate if intubated with water after lesions in this lateral area. These two findings form the only evidence available for the rat indicating that the structures involved are functionally and anatomically discrete. Andersson and McCann (8, 9) appear to have obtained a pure drinking response on electrical stimulation of a corresponding area in the hypothalamus of the goat. These workers (10) also have obtained adipsia or hypodipsia in the dog after lesions involving the lateral hypothalamic area, and noted that the dogs would, however, drink milk or broth although they refused water. The lesions in these experiments, however, extended right up to the third ventricle medially and so probably also destroyed the ventro-medial nucleus. Both in this work and in the stimulation responses described by Delgado and Anand (11) in the cat, the use of a fluid food creates a confusion between food and water which makes interpretation difficult.

The acute response of rats to full sham operation in this region with almost complete aphagia and adipsia for the 1st post-operative day and partial effects for the second day is remarkable and, so far, unexplained. The immediate simulation of the effects found in operated animals is confined to those sham-operated rats in which the needle track passed through the hypothalamus in the lateral area and does not appear to be the result of its passage through the cortex. Rats which are in poor condition will show some degree of hypodipsia for variable periods from purely cortical lesions. The spatial limits over which this mechanical lesion is effective are being further determined. This response leads to the speculation that the effects are produced by damage to tracts rather than to nuclei, and that the effective region may be much more closely circumscribed than the size of the electrolytic lesions might suggest. That is further pointed out by the two animals in which the purely mechanical lesions produced permanent aphagia and adipsia. It is important, therefore, in experiments involving these lesions to use fully sham-operated animals as controls.

The high incidence of successes in these operations using the revised coordinates indicates that the effective region is more dorsal than the area suggested by Anand and Brobeck (2), but our lesions are larger. The extent of the lesions is, laterally, well outside the region conventionally designated as the hypothalamus, and well into the subthalamic and thalamic areas carrying large numbers of fiber tracts including those from the supra-optic and paraventricular nuclei. They do, however, destroy the areas described by Krieg (12) as the lateral hypothalamic nuclei. It is not possible, therefore, to state definitely at present that the functional foci of these lesions is strictly in the hypothalamus, but only in the lateral subthalamic area. Interruption of the last-mentioned tracts may be, in part, responsible for the degree of polydipsia observed after recovery in the escaped rats. This behavior of the escaped rats might be related to the association of inhibition of diuresis with polydipsia found by Andersson and McCann (9) on stimulation of the rostral end of their ‘drinking area’ in goats. However, as these rats did not lose weight on restriction of water intake as they would be expected to do in true diabetes insipidus, and did not return to polydipsic intake after return to ad libitum water, it would appear that either sudden recovery from lesions producing diabetes insipidus is possible, or that this polydipsia was not simply a reflection of renal water loss.

The question of escape is one which, at first sight, might be explicable on a basis of slightly misplaced lesions producing a response qualitatively similar to but quantitatively variable from that of sham-operated controls. The apparent increase in incidence of escapes in the intubated rats, however, forces further examination of the problem.

The sensation of water in the gullet and stomach might be able to re-establish the pathways involved in eating and drinking. This would imply that the lesions are primarily of a kind to interfere with motor or coordinative mechanisms in eating and drinking and not of a kind to render the animals insensible to the metabolic and sensory stimuli of hunger and thirst. The partial involvement of the pyramids in some of the lesions provides some support for this as a possible modus operandi of the lesions. However Anand and Brobeck (2) found that more lateral lesions (2.5 mm from the mid-line) which mainly damaged the
internal capsule and the optic tract had only a slight and transient effect on eating.

It can be proposed, however, and the authors incline to this view, that all animals could, in theory, recover from this operation given sufficient time. That is, the lesions may produce, fundamentally, an acute and not a chronic functional failure. In the case of the operated unintubated animals the chance of systemic recovery is low because the animal will reach the irreversible phase of dehydration and general debility before recovery of specific nervous function. In the case of the intubated rats the weight loss, after the initial fall, is lower, so that these animals are maintained in the reversible phase for a longer period and so are more likely to respond to a recovery of nervous function. It is probably safe to assume here that the difference in body weight loss between intubated and unintubated groups is predominantly a reflection of degree of hydration. This explanation is also suggested by Teitelbaum and Stellar's finding that animals maintained on a tube-fed diet for as long as 65 days will eventually escape.

From the impermanence of the effect of these lesions along with their very drastic initial effects, it seems possible that the lesions affect one of the hunger or appetite controls which is normally dominant, but that alternative pathways of control can eventually take over with only a small loss in efficiency. That there is some loss of efficiency is suggested by the slow response to food dilution and the fat preferences of escaped rats shown by Teitelbaum and Stellar (3). The finding of these workers that aphagic rats will eat special foods before general recovery of eating recalls Kennedy's (13) distinction of two forms of control of food intake, a primitive hunger mechanism and an appetite or discriminatory mechanism. A tentative identification of these lateral centres with Magoun's central facilitatory mechanism, as suggested by Strominger and Brobeck (15) would be consistent with the present observations, particularly the phenomenon of escape.

Strominger (16) and Bruce and Kennedy (17) have convincingly argued that this is a physiological response to the increased urinary elimination necessitated by the raised food intake. In the present experiments the fall in water intake can be regarded as again proportional to fall in food intake if both fall to zero, although, strictly, the ratio is now indeterminate. But the same physiological rationalization cannot be invoked as the requirement for water elimination does not fall to zero until catabolism falls to zero. It is important in all these water exchange phenomena to distinguish between changes produced primarily by some disruption of the water intake process and those which are primarily disruptions of the water elimination process and are merely reflected in drinking activity. The distinction between 'metabolic' and 'regulatory' disturbances, made in the study of hyperphagia (18), due, respectively, to errors in peripheral tissue metabolism and to errors in specialized brain structures, might profitably also be applied to disturbances in water intake.

REFERENCES