Localization of the Adrenergic Feeding System in the Rat Diencephalon

Abstract. *Injection of 6 micrograms of aqueous norepinephrine elicits eating only when it takes place at sites within a limited region of the rat brain. The distribution of effective sites coincides with that of systems connected to an extrahypothalamic pathway between the limbic forebrain and tegmental motor systems. It does not correspond to those parts of the lateral hypothalamus thought to control normal feeding.*

Local application of norepinephrine within the hypothalamus of the rat can elicit eating (*1–3*). Unexpectedly, the site which is most often effective does not appear to be within the lateral hypothalamic "appetite center" (*4*). I now report an experiment to map the boundaries of the area in which injected norepinephrine evokes eating. The distribution of effective sites within the diencephalon should reveal the anatomical relations between the part of the feeding system sensitive to norepinephrine and those parts affected by electrical stimulation and by lesions.

A needle (gauge 27) closed by a plastic cap and insert wire was implanted stereotactically into the left side of the brain of male albino rats (350 g) with the method described by Grossman (*1*). One week after surgery and once each day thereafter for several days, rats were given access to wet mash, and the caps of the cannulas were removed and replaced. Then tests at intervals of 2 to 4 days were made to determine whether eating was elicited when the needle was used to guide injections of norepinephrine. Before testing, rats were given wet mash in their home cages for 10 minutes and then fresh Purina Laboratory Chow and tap water for 30 minutes. The rats were injected either with 65 m*M* (−)-norepinephrine bitartrate (*5*) in 90 m*M* NaCl or with 155 m*M* NaCl. Norepinephrine was given in the first and fourth tests; saline was given in the intervening tests. A standard volume of 0.675 μl was delivered by a 10-μl syringe in a repeating dispenser (*6*) through a 34-gauge needle inserted into the guide cannula. After injection, the rat was returned to the home cage and left with access to chow and water. The amounts consumed were measured 1 hour later. The frequency distributions of chow intakes after individual injections of norepinephrine and saline are given in the lower graph of Fig. 1. The response characteristic of an injection placement was calculated by subtracting intakes after saline from intakes after norepinephrine. The frequency distribution of placement responses was multimodal (upper graph, Fig. 1). Only four placements showed responses with the mean difference in the range 0.6 to 1.19 g. A placement was considered effective if the mean chow intake in the hour after norepinephrine injections was at least 1.2 g greater than the intake after saline injections (*7*).

The placement of injections was varied among 57 rats (six successive groups of 6 to 12). Each group included one or two controls with the guide cannula aimed at the placement known to be virtually always effective (*4*). In the remaining subjects, the cannulas were aimed at points spaced at 0.5-mm intervals along the three stereotactic coordinates through this placement. In successive groups of animals, the cannulas were implanted continually further from the most effective area until I found boundaries beyond which injections of the standard dose were ineffective. In initial tests in each rat, the injecting needle was cut to a length which just reached to the bottom of the cannula. In 16 of the rats, further tests were made with the needle protruding 1, 2, or 3 mm beyond the bottom of the cannula.

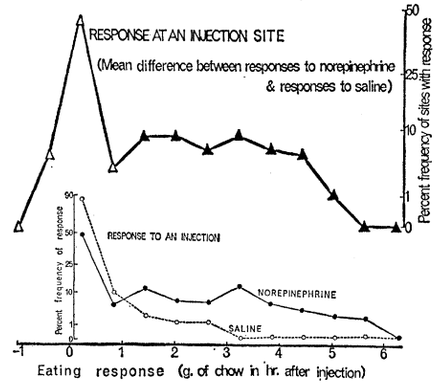

Fig. 1. Frequency distributions of eating responses after injections at various diencephalic sites. Frequencies are expressed as percentages of the total population, on scales with square-root intervals. Lower graph: eating responses to individual injections; upper graph: injection sites showing a given difference in eating response as the result of injection of norepinephrine or of saline. The responses whose frequencies are given by solid triangles constitute the effective sites in Fig. 2.

After each group had been tested, the brains were perfused and fixed in formalin, 25-μ frozen sections were cut, and every fourth one was stained with Luxol blue (*8*) or cresyl violet. Each injection site was located microscopically without knowledge of its effectiveness. When the needle protruded beyond the end of the guide cannula, the site could be seen as a necrotic ball of gliosis with a diameter of about 100 to 200 μ. The effectiveness of each needle placement was later correlated with its anatomical site (Fig. 2). The lateral, rostrocaudal, and dorsoventral limits of the region containing effective sites were evident, but the medial boundary was not as clear.

Occasionally, the distance between the edge of an effective site in one rat and an ineffective site in another was as little as 100 μ (see ventral placements in planes A7·8, A7·0, and A6·2, Fig. 2). The eating response is reliably elicited (*7*) and has all-or-none characteristics (Fig. 1). Therefore, there is reason to believe that this technique localizes to within about 100 μ the structures mediating the feeding response. The accuracy of this technique thus compares favorably with that obtained with small electrolytic lesions.

The control cannulas, implanted according to the coordinates most often effective (*4*), guided injections to the confluence of the stria medullaris (SM), lateral to the anterior hypothalamic area (AHA), namely, to those sites in plane A7·0 of Fig. 2 which are ventral to a depth of −1.7 mm and less than 2 mm lateral. This localization has been confirmed in 30 other rats not included in this series. Only the rostral edge of the region in which electrical stimulation can elicit hunger in the rat (*9*) contained sites at which norepinephrine evoked eating (planes A6·2 and A5·8, Fig. 2). More caudal portions of the electrically stimulated eating region did not mediate eating to norepinephrine, nor did the far lateral hypothalamic area (LHA) at the level of the ventromedial nucleus (VMH), a region in which bilateral lesions give anorexic aphagia (*10–12*). Injection of norepinephrine into these more caudal parts of the LHA did not produce side effects which could prevent eating. Thus, the results imply that the neural structures controlling feeding which re-

spond to the norepinephrine (*13*) are distinct from the structures in the lateral hypothalamus which were thought to integrate the control of food intake in normal hunger.

The boundary of the region containing effective injection sites is more restricted than that for drinking elicited by cholinergic drugs (*14*) even though fibers rich in norepinephrine have been seen at many of the ineffective sites (*15*). Sites at which norepinephrine elicited eating formed a coherent group extending through a part of the substantia innominata at the level of the AHA (see planes A7·0 to A6·2 in Fig. 2). They did not extend beneath the internal capsule (CI), contrary to indications from experiments with larger cannulas and doses (*2*). An unbroken sequence of effective sites spread dorsally from this grouping, both rostrally through nucleus accumbens septi (ACB) into the lateral septum (LS) and also caudally along the course of the SM and through adjacent rostral thalamic tissue to the ventral border of the lateral ventricle (V) rostral to the habenula (HL, HM). Although several effective sites were in or close to the ventricle (see planes A7·8, A7·4, and A5·4), other periventricular regions are probably not involved, since a number of ineffective sites were also approximately ventricular (see planes A9·4, A8·6, A7·8, and A5·0). Two smaller series of effective sites extended ventrally, one subfornically to the level of the rostral VMH (*16*) and the other toward the olfactory tubercle (TUO) at the ventral edge of the preoptic area (POA). The distribution of effective and ineffective sites was not changed when the cannulas were implanted in rats in which the contralateral LHA had been destroyed by an electrolytic lesion (six placements marked *L*, Fig. 2) or when different structures were damaged dorsally to the injection site by implanting the cannula at an angle (five placements marked by arrows, Fig. 2).

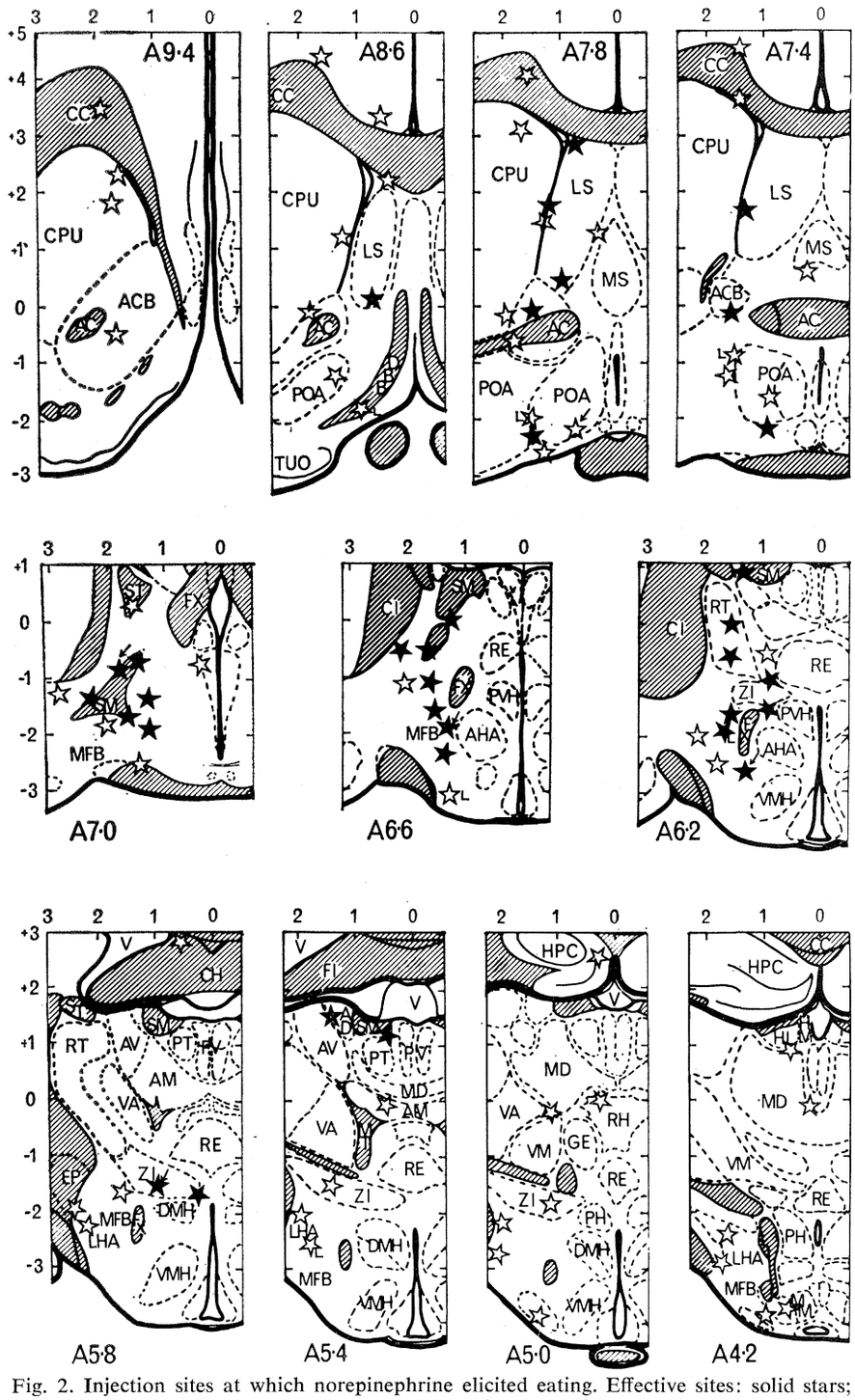

Fig. 2. Injection sites at which norepinephrine elicited eating. Effective sites: solid stars; ineffective sites: open stars. Where two overlapping sites give the same behavior, only one is plotted. *L*, implant in a rat with a contralateral LHA lesion. Arrow above a site indicates that the placement was approached at an angle from the right-hand hemisphere. Projected on planes at intervals of 0.4 or 0.8 mm as in the atlas of de Groot (*29*). For abbreviations, see text and (*29*).

Probably, connections still undetected by studies of degeneration are functionally very important in this region (*17*); hence, any identification of the adrenergic feeding system with known structures must be very tentative. Moreover, a number of diencephalic regions were not adequately sampled in this experiment. Nevertheless, except for some of the most caudal sites, there is a striking coincidence between the anatomy of the stria medullaris and the pattern of the structures implicated in eating elicited by norepinephrine. The lateral septum is one of the tract's origins (*18*). It has connections coming from the amygdala and TUO (*19*) and going to the anterior hypothalamus (*20*). The stria and related fibers (which may account for effective thalamic sites caudal to the SM) course through the rostral thalamus to the border of the lateral ventricles and there enter the habenular nuclei (*17, 20, 21*). These nuclei project into the mesencephalon (*20*) where they may influence motor systems which are necessary for feeding (*22*) and which

can mediate feeding reflexes independently of the hypothalamus (*23*). Therefore, injected norepinephrine may excite or inhibit a part of the feeding system which passes between the limbic forebrain and the midbrain along an epithalamic route which lies parallel to the well-established lateral hypothalamic connection (*17*, *24*) and may be cross-linked to it by transthalamic fibers (*12*, *17*). This postulated part of the feeding system may be involved in the hyperphagia which results from some lesions in the dorsal thalamus or stria medullaris (*25*). The epithalamic pathway might also mediate the recovery from aphagia shown by rats with lateral hypothalamic lesions: indeed the feeding patterns of such rats show abnormalities (*11*) similar to those associated with feeding elicited by norepinephrine (*3*, *26*). In both cases, the rats are hypersensitive to the flavor of food and unwilling to expend muscular effort to obtain it.

A search should be made ipsilaterally to effective injection sites for areas which, when destroyed, eliminate the eating elicited by norepinephrine. Such experiments would both test the scheme outlined above and clarify the afferent and efferent connections (*18*, *19*, *21*, *24*) of this rostral hypothalamic part of the feeding system. As axonal uptake mechanisms and alpha adrenergic receptor sites mediate the elicitation of eating by norepinephrine injected into the substantia innominata (*27*), connections with adrenergic systems related to that area (*2*, *28*) are of particular interest. Whether the adrenergic portion of the feeding system is afferent, efferent, or in parallel to the lateral hypothalamic portions of the system, its role in normal hunger remains to be determined.

DAVID A. BOOTH

School of Biological Sciences,
University of Sussex, Brighton, England

References and Notes

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4. This was suggested by results from rats prepared for other experiments (*3*, *27*), in which two sets of coordinates were used for implantation of cannulas. Only 16 of 33 cannulas in 25 rats were effective in the test of eating after injection of norepinephrine (detailed in the text) when the cannulas were aimed at coordinates recommended for electrical stimulation of eating (*9*). Cannulas aimed 0.8 mm more anteriorly were effective in 59 of 63 cases in 54 rats. This locus was 8.0 mm below the upper surface of the skull when the cannula was lowered vertically through the bregmoidal suture 1.3 mm laterally, with the incisor bar 3.5 mm above the interaural line.
5. The hydrochloride was equally effective (*27*), but the bitartrate was the purest available salt.
6. Hamilton Co., Whittier, California.
7. Intakes of chow after norepinephrine were consistently large with repeated injections (range for all tests of effective cannulas: 0.8 to 4.7 g; median difference between first and second norepinephrine injections: 0.8 g). Water intakes were small—often nil—at most 5 ml in an hour after injection. A choice between water and relatively unpalatable dry chow was given to increase the strength of data on food intake as evidence for effects on a feeding system rather than less specific oral effects.
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13. The elicitation of eating is unlikely to be secondary to stimulation of the heat-regulating system in the anterior hypothalamus, as that is more sensitive to epinephrine than to norepinephrine [W. Feldberg and R. D. Myers, *J. Physiol. London* **177**, 239 (1965)], unlike the feeding system (*27*). Also, carbamyl choline injected into this region in the rat elicits drinking but no eating [D. Quartermain and N. E. Miller, *J. Comp. Physiol. Psychol.* **62**, 350 (1966)] and yet causes greater hypothermia than does norepinephine [N. E. Miller, *Science* **148**, 328 (1965)].
14. Unlike cholinergic elicitation of drinking [A. E. Fisher and J. N. Coury, *Science* **138**, 69 (1962)], injection of norepinephine into Papez' circuit structures consistently failed to elicit eating under the present conditions (see Fig. 2), although 1 to 3 μg or more of solid norepinephrine in these and all tested structures occasionally causes augmented intake of foodstuff which is also eaten under control conditions [J. N. Coury, *Science* **156**, 1763 (1967)]. The pattern of effective sites in Fig. 2 also indicated that the drug could not merely be acting directly on VMH structures to inhibit satiety [J. Olds, A. Yuwiler, M. E. Olds, C. Yun, *Amer. J. Physiol.* **207**, 242 (1964)].
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16. The two effective sites in the zona incerta (ZI) might be related to inferior thalamic projections to dorsal thalamus, which possibly mediate eating stimulated electrically [O. A. Smith, W. L. McFarland, H. Teitelbaum, *J. Comp. Physiol. Psychol.* **54**, 484 (1961)]. The distribution of effective placements was generally more caudal in earlier work [S. P. Grossman, *Amer. J. Physiol.* **202**, 872 (1962)] in which cannulas over 0.8 mm wide were used to place crystalline norepinephrine which gives more extensive necrosis than its aqueous solution does [J. Sutin, L. Van Orden, T. Tsubokawa, in *EGG and Behavior*, G. H. Glaser, Ed. (Basic Books, New York, 1963)]. Even when solutions are used, such guide cannulas are associated with lesions over 1 mm wide (*26*). The implant at the angle at which it is usually placed damages anterodorsal structures (in the planes of Fig. 2); hence, the larger the cannula, the more apt there is to be a misleading displacement of the effective sites. Furthermore, a comparison of the dose-response curves for large and small cannulas (*27*) indicates that tissue damage facilitates low intensity eating responses to norepinephrine while lowering the maximum response elicitable. Size of the cannula is therefore critical for useful anatomical studies.
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30. Most of this work was done during appointments at Yale and Rockefeller universities supported by PHS grant MH 02949 to Professor N. E. Miller. I thank G. Ogren, N. Cox, Miss E. Sherwood, Mrs. J. Hogan, Mrs. E. Love, Mrs. A. Wechsler, and Mrs. T. Craig for technical assistance. The microscopy was performed at the University of Sussex on a U.K. Science Research Council fellowship. I thank Drs. T. S. Collett and G. Wolf for advice.

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Motivated Forgetting Mediated by Implicit Verbal Chaining: A Laboratory Analog of Repression

Abstract. *After learning an* A-B *paired-associates list, college students read a list of* D *words, several of which were consistently accompanied by unavoidable electric shock. The* D *words were members of implicit* B-C, C-D *chains, inferred from published word-association norms. In a subsequent recall test of the original* A-B *list, the* B *words that were implicitly associated with the shocked* D *words were forgotten significantly more often than control words.*

Are memory items which are specifically associated with unpleasant events more readily forgotten than affectively neutral items? Despite the wealth of empirical and theoretical interest in this question, particularly with respect to the psychodynamic concept