the eye closure, and in the other (MD4) on the right, ipsilateral to the deprived eye. In all three kittens, in the nondeprived laminae, there was no significant difference between the mean size of the total cell population and that of the marked cells. However, the marked cells in the deprived laminae were from 14 to 28 percent smaller than those in the nondeprived laminae. A comparison of the histograms of cell size (Fig. 1, C and D) shows a somewhat higher peak of the small cells in the deprived laminae, compared with a broader, flatter distribution in the normal laminae.

The results of cell measurements in the LGN on the side of the area 18 injection are very different. HRP was injected into the right area 18, ipsilateral to the lid suture in MD1 to MD3; the injection was on the left in MD4. In the LGN ipsilateral to the area 18 injection, labeled cells from the nondeprived laminae were up to 26 percent larger than the mean of all cells measured, but in the deprived laminae labeled cells were of similar size to the mean. Comparison of the size of marked cells between the normal and the deprived laminae revealed a striking difference, of from 50 to 64 percent, and the histograms (Fig. 1, E and F) show a very clear shift from a wide, relatively flat distribution in the undeprived laminae to a sharply peaked distribution entirely within the small cell population in the deprived laminae. The "shrinkage" of these presumed Y cells, marked by injection of HRP in area 18, is much more than that of the neurons labeled after injection in area 17, most of these cells probably being X cells. In fact, inspection of Table 1 shows that, in those animals where both sides were successfully injected, labeled Y cells are 12 to 28 percent smaller than labeled X cells in the deprived laminae (*13*).

We draw the following conclusions. In a normal adult cat, HRP injected into area 18 reaches a greater proportion of the large neuronal somata in the LGN than when injected into area 17. There are good physiological and anatomical data to permit identification of the largest neurons of the LGN with the fast-conducting Y system projecting mainly to area 18. When HRP is injected in area 18, one can, therefore, postulate that the Y cells of the LGN on the side injected will be labeled. We have shown here that, in monocularly deprived kittens, reaction product is restricted to very small cells of the deprived laminae, which can be taken to represent the diminished Y cells. When the injection is in area 17, which should cause predominant labeling of X cells, the marked neurons in the deprived laminae are smaller than those in the nondeprived laminae but to a much lesser degree. Thus, the present experiments provide morphological evidence that monocular deprivation in cats does have a relatively specific effect on the Y cell system.

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11. C. D. Gilbert and J. P. Kelly, *J. Comp. Neurol.* **163**, 81 (1975). Detailed comparison between the results of these authors and our own data is not easy for several reasons. (i) Gilbert and Kelly appear to have used different animals for injections into areas 17 and 18, thereby introducing large differences in mean cell size between samples. (ii) In their experiments a much larger fraction of measured LGN neurons was labeled after an area 17 injection, but they state that they may have excluded very small cells, probably interneurons, which we did not. (iii) There are some numerical inconsistencies in their paper which make intrepretation difficult.
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13. The design of the experiment necessitates comparison of lamina A on one side with A1 on the other. While this approach overcomes the problems associated with comparisons between animals [see (*11*)], it is only justified if the overall distribution of cells is similar in the two laminae being compared. In fact, the shape of the distribution and mean cell size was much the same in the two laminae.
14. Supported by grants from the Fonds National Suisse de la Recherche Scientifique (3/2460/74), the Medical Research Council, London (G/972/463/B), and the European Training Programme in Brain and Behaviour Research. C.B. held a fellowship from the Roche Research Foundation during a visit to Lausanne. We thank M. Gissler and M. Dürsteler for computer programming, and M. C. Cruz, R. M. Cummings, and B. Rhodes for technical help.

6 July 1976; revised 6 October 1976

Selective Blockade of Hypothalamic Hyperphagia and Obesity in Rats by Serotonin-Depleting Midbrain Lesions

Abstract. *Adult female rats, depleted of 70 percent of forebrain serotonin by dorsal and median raphe lesions, showed little overeating of food pellets and obesity following medial hypothalamic lesions. However, these rats showed the same reduced acceptance of sucrose solutions, enhanced rejection of quinine solutions, and exaggerated weight gain on a high-fat diet as did other rats made obese by medial hypothalamic lesions alone. Since raphe lesions alone produced none of these effects, the pattern of behaviors observed suggests a hitherto unknown (perhaps secondary) role for brain serotonin metabolism in selective aspects of the medial hypothalamic syndrome.*

It has been estimated that 20 to 40 percent of all North Americans are obese (*1*). Considering the known health risks of excessive weight, it is not surprising that many animal models have been studied to understand its pathophysiology. The most prominent model employs rats with medial hypothalamic (MH) lesions. The marked behavioral similarities between the obese human and this experimental analog (*2*) suggest common underlying disorders.

The complex behavioral changes attendant upon MH injury imply alterations to multiple neuronal systems (*3*). Efforts to define these systems have recently focused on the role of monoamines (*4, 5*). Several reports indicate that damaged hypothalamic or forebrain (or both) serotonin (5-hydroxytryptamine or 5-HT) systems contribute to MH hyperphagia and obesity (*6*). Seemingly incompatible with this suggestion are findings that chronic 5-HT depletion by midbrain raphe lesions does not elevate food intake and body weight (*7*). We present evidence clarifying this paradox, namely, that such raphe damage can largely prevent the overeating and obesity characteristic of MH injury. These findings imply a secondary rather than a primary role for 5-HT in MH hyperphagia.

Forty-two adult female rats (Wistar strain, High Oaks Ranch, Ontario) were individually housed in a colony with light (0800 to 2000 hours) and temperature (22° ± 1°C) controlled and given free access to weighed amounts of Purina food pellets (on cage floor) plus tap water (in 100-ml Wahmann bottles). Half had received dorsal and median raphe lesions

and the other half sham surgery 50 days previously (*8*). Bilateral MH lesions in 12 rats that had received sham surgery (*9*) produced the prompt and marked eating and weight gain traditionally associated with such brain injury (Table 1A). In the first postoperative week rats with sham + MH lesions ate an average of 56.7 percent more food than eight sham + sham controls and gained almost 19 percent above their weight at operation (for both increases, $P < .01$) (*10*). This phase of hyperphagia subsided by the third or fourth week, culminating in net weight gains of 40 percent above control levels by the seventh week ($P < .001$). Contrary to this pattern, MH lesions in 12 rats with prior raphe lesions produced first-week feeding increments (+12.5 percent) or weight gains (+6.0 percent) that were not statistically different from those seen in nine raphe + sham controls. By the end of 7 weeks, rats with raphe + MH lesions had attained somewhat greater ($P < .05$) weight gains than raphe + sham controls. However, these gains were considerably less ($P < .01$) than those seen in rats with sham + MH lesions. As expected (*7, 8*), raphe + sham lesions did not induce overeating and weight gain. Water intakes of all groups paralleled food intakes. This first experiment demonstrated that midbrain raphe lesions, which themselves do not modify long-term feeding and weight regulation, can substantially block MH hyperphagia and obesity.

To determine if other consummatory behaviors were altered in rats with raphe + MH lesions, we first replaced each animal's water with one of six sucrose and then four quinine solutions consecutively while food pellets were still available. The sequence and concentrations of solutions presented were 1, 2, 4, 8, 16, and 32 g of sucrose in 100 ml of water, then 10, 5, 2.5, and 1.25 mg of quinine hydrochloride in 100 ml of water. Each solution was presented for 24-hour intake tests with tap water available for 48 hours between presentations. Following these measures, rats were given tap water and allowed 1 week to reestablish baseline intakes, and then their food pellets were replaced for 24 days with a high-fat diet (33 percent Crisco vegetable shortening plus 67 percent Purina powdered food pellets) placed in glass cups inside the cages. If an intact midbrain raphe axis were required for the depressed acceptance of sucrose solutions (*5, 11*), enhanced rejection of quinine solutions (*5, 12*), and exaggerated weight gain on high-fat diets (*13*) characteristic of the MH syndrome, attenuation of these intake patterns would be expected for rats with raphe + MH lesions.

There were no differences in cumulative intakes of sucrose or quinine solutions between the raphe + MH and sham + MH groups (Table 1B). As expected (*5, 11*), sham + MH rats showed reliably suppressed sucrose and quinine intakes compared to sham + sham controls (one-tailed $P < .05$ and $P < .01$, respectively). The capacity of MH lesions to attenuate sucrose acceptance was also observed in raphe + MH rats compared to raphe + sham controls ($P < .001$). Similarly, quinine rejection tended to be greater ($P < .10$) for raphe + MH rats. Of added interest, rats with raphe + sham lesions showed enhanced sucrose acceptance and quinine rejection ($P < .05$ for both) compared to rats with sham + sham lesions. These findings indicate that the altered acceptance of sucrose and quinine solutions after MH injury are independent of the midbrain raphe axis. Nevertheless, this locus may exert separate influences over mechanisms mediating ingestion of these solutions.

Prior raphe destruction also did not attenuate the exaggerated weight gain of rats with MH lesions fed a high-fat diet

Table 1. (A) Food intake and body weight gains for all four groups either 7 or 49 days after MH lesion or sham surgery. Rats were fed standard Purina pellet chow. Two groups had received dorsal and median raphe lesions 50 days before MH surgery. The group with raphe + MH lesions showed a lower food intake and lower weight gain than the group with sham + MH lesions. (B) Cumulative intakes of six sucrose and four quinine solutions (see text). Each solution replaced tap water for 24 hours with a return to water for 48 hours between tests. Despite their lack of obesity, rats with raphe + MH lesions showed intakes of both types of solutions similar to those of obese rats with sham + MH lesions. (C) Body weights of all four groups before and after 24 days in which food pellets were replaced with a high-fat diet (see text). Again, despite lack of obesity, rats with raphe + MH lesions showed exaggerated weight gain comparable to that of obese sham + MH rats. All data are expressed as group means ± standard errors of means (*10*).

Parameter	Group (*N*)			
	Raphe + MH (12)	Sham + MH (8)	Raphe + sham (9)	Sham + sham (8)
	A. Food intake and body weight after MH surgery			
Operation weight (g)	250 ± 4.97	267 ± 4.42	235 ± 7.76	250 ± 5.24
Food intake, 1 week (g)	13.1 ± 1.51*†	20.2 ± 2.16‡	11.6 ± 1.08§	12.9 ± 1.07
Increase above operation weight, day 7 (g)	14.6 ± 5.73*†	50.3 ± 6.56‡	1.11 ± 1.92§	−1.00 ± 2.81
(Change, %)	(+5.84 ± 2.29)	(+18.8 ± 2.46)	(+0.47 ± 0.82)	(−0.40 ± 1.12)
Food intake, 7 weeks (g)	14.8 ± 1.74*‖	22.6 ± 2.14‡	14.3 ± 1.31§	16.8 ± 1.02
Increase above operation weight, day 49 (g)	62.3 ± 14.8¶†	139 ± 19.4#	23.7 ± 3.33§	31.8 ± 3.67
(Change, %)	(+24.9 ± 5.92)	(+52.1 ± 7.27)	(+10.1 ± 1.42)	(+12.7 ± 1.47)
	B. Sucrose and quinine acceptance			
Three-day mean baseline water intake (ml)	34.3 ± 3.71	41.7 ± 7.41	31.1 ± 2.40	28.6 ± 1.27
Cumulative sucrose intake above baseline (ml)	+401 ± 34.2#	+332 ± 71.1*	+669 ± 34.2‖	+523 ± 65.6
Cumulative quinine intake below baseline (ml)	−111 ± 14.8**§	−124 ± 25.3¶	−79.1 ± 8.01‖	−55.6 ± 6.17
	C. Body weights before and after 24 days of high-fat diet			
Starting weight (g)	318 ± 14.6	441 ± 34.4	270 ± 8.94	301 ± 6.96
Day 24 weight (g)	476 ± 32.7	611 ± 39.7	330 ± 16.8	352 ± 12.9
Weight change (g)	+158 ± 20.1‡§	+170 ± 7.79‡	+60 ± 8.77§	+51 ± 8.61

*Not significant (NS) compared to same-first operation (SFO) + sham control. †$P < .01$ compared to sham + same-second-operation (SSO) control. ‡$P < .01$ compared to SFO + sham control. §NS compared to sham + SSO control. ‖$P < .05$ compared to sham + SSO control. ¶$P < .05$ compared to SFO + sham control. #$P < .001$ compared to SFO + sham control. **$P < .10$ compared to SFO + sham control.

Table 2. Chronic effects of raphe, or raphe + MH lesions on forebrain monoamine or metabolite concentrations. One rat with sham + MH lesions died before monoamine determinations. The remaining rats were killed 205 days after raphe lesions (155 days after MH lesions). All values are given in nanograms per gram of fresh tissue and expressed as means ± standard errors of means (*10*).

Group (*N*)	Compound measured			
	5-HT	5-HIAA	NE	DA
Raphe + MH (12)	192 ± 14.0*	75.7 ± 5.56*	306 ± 19.3*	1536 ± 33.4†
Sham + MH (7)	663 ± 15.6‡	264 ± 7.44§	340 ± 12.6‖	1470 ± 52.3§
Raphe + sham (9)	171 ± 11.2*	76.3 ± 11.2*	344 ± 10.9¶	1580 ± 36.2†
Sham + sham (8)	705 ± 15.8	266 ± 8.49	397 ± 7.38	1530 ± 39.6

*$P < .001$ compared to sham + same-second-operation (SSO) control. †Not significant (NS) compared to sham + SSO control. ‡$P < .10$ compared to same-first-operation (SFO) + sham control. §NS compared to SFO + sham control. ‖$P < .05$ compared to SFO + sham control. ¶$P < .05$ compared to sham + SSO control.

(for both MH groups $P < .01$ compared to controls that had received the same first operation) (Table 1C). Indeed, both MH groups gained the same weight ($P < .20$ between groups). Much as with the sucrose and quinine data, these findings show MH reactivity to a palatable diet to be independent of the midbrain raphe axis.

Monoamine assays of endogenous 5-HT, its major metabolite 5-hydroxyindoleacetic acid (5-HIAA), norepinephrine (NE), and dopamine (DA) were performed on forebrains of all rats who completed behavioral testing (*14*). The major effect of raphe lesions (Table 2) was to profoundly deplete (−70 percent) 5-HT and 5-HIAA (all $P < .001$ for comparisons between either raphe group and its control group that had received the same second operation). Small (10 to 13 percent) but reliable ($P < .05$) decrements in NE were also observed for these groups. Dopamine levels remained unchanged (*15*). As anticipated (*5, 6*), sham + MH lesions reduced forebrain NE and 5-HT (one-tailed $P < .025$ and $P < .05$, respectively, compared to sham + sham controls) without affecting 5-HIAA or DA.

The exact mechanism by which midbrain raphe lesions can attenuate MH hyperphagia is unknown. Conceivably, some of our findings could reflect damage to other, as yet unknown, neural systems coursing through the central gray. This seems unlikely in view of the apparent specificity of our lesions (*8, 15*) (Table 2). Since raphe lesions primarily impair 5-HT neurotransmission (*16*), the attenuated obesity observed—which was not completely compensated for even with access to a high-fat diet—suggests an important role for 5-HT in mechanisms mediating weight regulation after MH injury (*6*). The probability that our findings relate only to the small NE depletions observed after raphe injury seems remote; chronic depletion of 80 percent NE and DA with 6-hydroxydopamine does not block MH hyperphagia but does diminish quinine rejection (*5*).

It is possible that altered 5-HT and NE transmission together can explain this reduced obesity after raphe + MH lesions. Comparisons (*10*) between the weight gain by these rats 1 week after MH damage and all assay results revealed significant correlations only with 5-HT and 5-HIAA levels ($r = .6055$ and $.6078$, respectively; $P < .05$ for both). Similar correlations between week 7 weight gains and assay results revealed significance only with NE levels ($r = -.6871$; $P < .02$). The latter finding agrees with previous suggestions of inverse relationships between MH obsesity and brain NE (*4, 6*). No such correlations were found between weight changes and assay results for other groups. Therefore, the magnitude of MH obesity may be limited initially by impaired 5-HT metabolism, but with time this effect is partially compensated for (*17*), so that altered NE metabolism associated with MH injury predominates and permits minimal overeating. This possibility, and the fact that raphe lesions alone do not enhance feeding and weight gain, imply a secondary (modulatory) rather than primary (sensor or effector) role for 5-HT in MH hyperphagia.

Our data provide important new evidence for a significant role of 5-HT in mediating selective components of MH pathology. The interactive nature of the effects reported on weight regulation but not acceptance behaviors suggests two cautionary notes. First, the exclusive use of highly preferred foodstuffs to maintain experimental animals can conceivably obscure results of studies designed to differentiate among neural systems contributing to model feeding pathologies. Second, by analogy with these and other (*2*) animal findings, treatment of the obese human's weight exclusive of his altered food preferences (*18*) may invite resistance to or even reversal of weight reduction.

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2. S. Schachter and J. Rodin, *Obese Humans and Rats* (Wiley, New York, 1974).
3. Aside from overeating and obesity, MH damage can produce overreactivity to palatable and unpalatable foodstuffs, irritability, hypoactivity, and altered copulatory behaviors; studies employing knife cuts [A. Sclafani, *J. Comp. Physiol. Psychol.* **77**, 70 (1971); G. Paxinos and D. Bindra, *ibid.* **79**, 219 (1972); *ibid.* **82**, 1 (1973)] suggest that the neural substrates of these behaviors differ.
4. For example, impaired noradrenergic (NA) transmission has been implicated in MH hyperphagia [J. E. Ahlskog and B. G. Hoebel, *Science* **182**, 166 (1973); R. M. Gold, *ibid.*, p. 488; S. D. Glick, S. Greenstein, D. H. Waters, *Pharmacol. Biochem. Behav.* **1**, 591 (1973); G. Kapatos and R. M. Gold, *ibid.*, p. 81; J. E. Ahlskog, *Brain Res.* **82**, 211 (1974)]. However, an exclusive NA role in such hyperphagia seems unlikely since (i) selective NA disruption is neither necessary nor sufficient to induce overeating [J. Lordin, G. A. Oltmans, D. L. Margules, *J. Comp. Physiol. Psychol.* **90**, 144 (1976)], (ii) large, long-term depletion of forebrain catecholamines does not block MH hyperphagia (*5*), and (iii) the hyperphagia after MH lesions is additive to that following NA nerve damage [J. E. Ahlskog, P. K. Randall, B. G. Hoebel, *Science* **190**, 399 (1975)].
5. D. V. Coscina, C. Rosenblum-Blinick, D. D. Godse, H. C. Stancer, *Pharmacol. Biochem. Behav.* **1**, 629 (1973).
6. Notably, electrothermic MH lesions in rats [(*5*); D. V. Coscina, D. D. Godse, H. C. Stancer, *Behav. Biol.* **16**, 365 (1976)] and systemic injection of gold thioglucose in mice (D. V. Coscina, D. D. Godse, H. C. Stancer, in preparation) produce obesity while depleting forebrain 5-HT and norepinephrine. In rats with lesions, the degree of weight gain is inversely correlated with levels of 5-HT, its major metabolite, and norepinephrine, all of which are positively correlated with each other. Perhaps related to these findings is a recent report that transient depletion of brain 5-HT by ventricular injection of *p*-chlorophenylalanine produces transient hyperphagia and weight gain [S. T. Breisch, F. P. Zelman, B. G. Hoebel, *Science* **192**, 382 (1976)]. However, the sustained hyperphagia reported after 5-HT depletion with central 5,7-dihydroxytryptamine injection seems to support growth rather than fat deposition and is less clearly related to MH hyperphagia [C. F. Saller and E. M. Stricker, *Science* **192**, 385 (1976)].
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8. Both raphe nuclei send projections to or through the hypothalamus [L. C. A. Conrad, C. M. Leonard, D. W. Pfaff, *J. Comp. Neurol.* **156**, 179 (1974); S. A. Lorens and H. C. Guldberg, *Brain Res.* **78**, 45 (1974); B. L. Jacobs, W. D. Wise, K. M. Taylor, *ibid.* **79**, 353 (1974); P. Bobillier, F. Petitjean, D. Salvert, M. Ligier, S. Sequin, *ibid.* **85**, 205 (1975)]. Therefore, both received lesions to maximize 5-HT depletion. With the incisor bar 2.5 mm below the interaural line, radio-frequency heat lesions were produced under Nembutal anesthesia by generating 55°C for 1 minute at stereotaxic coordinates anteroposterior (AP), −0.35 mm; medial-lateral (ML), 0 mm; and dorsoventral (DV), −5 and −7 mm [L. J. Pellegrino and A. J. Cushman, *A Stereotaxic Atlas of the Rat Brain* (Appleton-Century-Crofts, New York, 1967)]. Histologic

verification of lesion sites at the end of testing revealed tissue damage 1.2 to 1.5 mm in diameter restricted to both raphe nuclei and the tissue rostral to them. These lesions produced anorexia and hyperdipsia in the first postoperative week, as previously reported (*7*). By the time of MH surgery, food intake, water intake, and weight gain were normal.

9. With heads flat between lambda and bregma skull sutures, 55°C heat lesions (*8*) were produced in each hemisphere for 1 minute at AP, +5.5 mm; ML, 0.5 mm; and DV, −8.6 to −9.0 mm. Lesions were 2.5 mm in diameter and 3.0 mm in length, extending from the anterior hypothalamus to the posterior ventromedial nuclei. Three rats with sham + MH lesions ate so voraciously that they suffocated within 48 hours of surgery; one sham + sham and one other sham + MH rat died from congestion during anesthesia.
10. All statistical comparisons were based on *t*-tests or Pearson product moment correlations. Probabilities reported are two-tailed unless predicted by previous results and indicated as one-tailed.
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14. Several other behavioral tests made on all rats will be reported elsewhere (D. V. Coscina, R. A. McArthur, H. C. Stancer, in preparation). Forebrain tissue was obtained, prepared, and analyzed by fluorometric assays as described before (*5*).
15. Radioenzymatic assays (D. V. Coscina and K. Lloyd, in preparation) of choline acetyltransferase and glutamic acid decarboxylase activities in seven brain regions from eight other rats with raphe lesions and six normal controls revealed no substantial differences. These data further support the specificity of our raphe lesions to forebrain 5-HT neurons.
16. M. J. Kuhar, R. H. Roth, G. K. Aghajanian, *Brain Res.* **35**, 167 (1971); *J. Pharmacol. Exp. Ther.* **181**, 36 (1972); M. J. Kuhar, G. K. Aghajanian, R. H. Roth, *Brain Res.* **44**, 165 (1972).
17. We recently found that 10 days after dorsal and median raphe lesions, 5-HT turnover following injection of ^{14}C-labeled 5-hydroxytryptophan is increased [J. J. Warsh, D. V. Coscina, H. C. Stancer, *Brain Res. Bull.* **1**, 273 (1976)]. Conceivably, the additional destruction of 5-HT neurons after MH lesions (*6*) would require some time to permit similar compensatory changes in the face of existing metabolic demands on residual neurons. At the same time, the sensitivity of postsynaptic 5-HT receptors might change with time [see Y. Agid, F. Javoy, J. Glowinski, *Nature (London)* **245**, 150 (1973) for a similar suggestion concerning less than totally destroyed DA neurons]. To add to these complexities, NE neurons damaged by MH lesions (*4–6*) could show similar time-dependent changes. Such changes, acting alone or in concert with 5-HT changes, might eventually permit reduced MH hyperphagia and weight gain.
18. Even after substantial weight loss, once-fat individuals display aberrant food preferences characteristic of the obese (*2*, p. 83).

19. This work was supported by the Clarke Institute of Psychiatry. We thank R. McArthur, P. Chan, and M. Guttman for technical assistance. J. Warsh and P. Garfinkel read earlier versions of this report.

12 July 1976

Endogenous Opiate Receptor Ligand: Electrically Induced Release in the Guinea Pig Ileum

Abstract. *Opiate receptors mediate the electrically evoked inhibition of the myenteric plexus–longitudinal muscle preparation of the guinea pig ileum. The electrically induced activation of the opiate receptor was produced by a prolonged simulation at 10 hertz and provides the first evidence that an endogenous opiate receptor ligand is released by nerve stimulation. The specificity of the phenomenon was demonstrated by the reversal obtained with the narcotic antagonists naloxone, naltrexone, and GPA 1843; GPA 1847, the (+)-isomer of 1843, did not cause reversal. The model system described should be useful for the study of the storage, synthesis, and release of endorphins.*

Although endorphins (*1*), which are endogenous opiate-like peptides (*2, 3*), and opiate receptors (*4, 5*) are found in the central and peripheral nervous systems, the effects of opiate antagonists described so far are surprisingly few and of small magnitude. It has been reported that the reaction time to nociceptive stimuli can be reduced by the narcotic antagonist naloxone (*6*). These observations suggest that endorphins may have an inhibitory effect on the neuronal pathways that mediate nociceptive effects. The effects of narcotic antagonists have not yet been demonstrated on the electrically induced contractions of the myenteric plexus–longitudinal muscle preparation of the guinea pig ileum; however, this tissue is known to be exquisitely sensitive to opiates (*7, 8*), to bind stereospecifically labeled narcotic agonists and antagonists (*4, 8*), and to contain enkephalin (*3*). The only effect that opiate antagonists have on this preparation has been described by Waterfield and Kosterlitz (*9*), who have demonstrated that naloxone produces a small increase in the output of acetylcholine evoked by field stimulation.

Since the effects of opiates and exogenous endorphins on the electrically stimulated guinea pig ileum can only be demonstrated at very low frequencies of stimulation such as 0.1 to 0.017 hertz (*10*), most of the studies on the effects of naloxone on this system have been carried out at low frequencies. We thought that the optimal frequency for the release of endorphins might be higher than 0.1 hertz, and therefore that the peptide release might not be apparent when the frequency of stimulation is optimized to demonstrate its opiate-like effects.

In attempts to provide evidence for the release of endorphins from the guinea pig ileum, we combined periods of stimulation at different frequencies. Initial experiments demonstrated that a naloxone-sensitive inhibition of the contractions of the longitudinal muscle could be elicited after periods of stimulation at 10 hertz.

The myenteric plexus–longitudinal muscle strip was prepared as described by Paton and Zar (*11*) and Kosterlitz *et al.* (*12*). Each strip was suspended in a 10-ml organ bath containing Krebs-bicarbonate solution at 37°C and gassed with a mixture of 95 percent O_2 and 5 percent CO_2; the composition of the Krebs solution differed from that previously described in that it contained $3 \times 10^{-5} M$ choline chloride (*13*). The isometric contractions of the muscle were registered with a Grass force transducer (model FT 03C) coupled to a Grass polygraph; the tension of the strip was maintained at 0.3 g. The tissue was stimulated through two platinum ring electrodes with supramaximal rectangular pulses of 1-msec duration applied at a frequency of 0.1 hertz. The opiate-like inhibition was elicited by a 5-minute stimulation of the same voltage and pulse duration but at a frequency of 10 hertz. The electronic equipment used has been described (*13*).

A marked inhibition of the basal contractions appears after the stimulation at 10 hertz (Fig. 1, top). The degree of inhibition is a function of the duration of the period of stimulation as well as frequency, voltage, and duration of the puls-

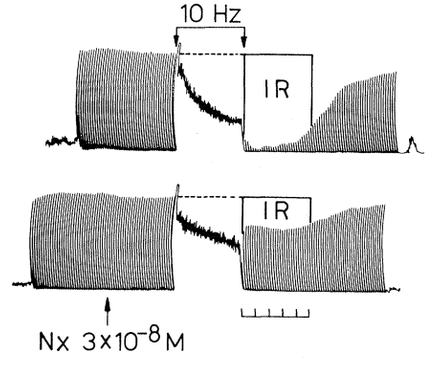

Fig. 1. Inhibitory response (IR) of the myenteric plexus–longitudinal muscle preparation elicited by stimulation at 10 hertz (top) and reversal by naloxone (bottom). The area of the recorded contractions generated during 5 minutes by stimulation at 0.1 hertz was measured before (basal response, BR) and immediately after stimulation at 10 hertz (post-stimulation response, PSR). The inhibitory response was calculated by subtracting the PSR from the BR. Naloxone (Nx), at the concentration indicated, was added 5 minutes before the stimulation at 10 hertz in order to measure the BR in the presence of the drug (the horizontal calibration is 1 minute per division).