were reconstructed from serial alternate sections. The HRP reaction product was distributed in two zones: a large, weakly stained area and a smaller, heavily stained area. There is evidence that the area of active uptake of HRP by axon terminals coincides with the densely stained zone (*10*). However, it should be noted that even the larger weakly stained area in our animals never spread caudal to the mesencephalic-diencephalic junction zone.

To date, we have analyzed only the segments of the spinal cord from the fifth lumbar level caudally. A total of 4439 cells (average of 555 cells per animal) have been found to contain label that was transported retrogradely from the diencephalon. By definition, all such cells contribute to the spinothalamic tract. Many of the cells (23 percent) were in the marginal zone (Fig. 1A), a region that is rich in neurons responsive to input from nociceptors (*11*). Another area in which spinothalamic cells were concentrated was the region equivalent to Rexed's lamina V (*6*), and there were also spinothalamic cells in the intermediate region and ventral horn. These observations confirm the physiological evidence of such a distribution of spinothalamic cells in work done in our laboratory (*12*) and anatomic evidence from the HRP studies of several groups (*13*). An unusual finding in our study, however, was the size of the population of marginal cells that project to the diencephalon. Many of these cells were small and were often located in Lissauer's fasciculus or the adjacent portion of the marginal zone. We attribute our success in demonstrating such a large number of spinothalamic cells in the marginal zone to the use of the *o*-dianisidine and tetramethylbenzidine reactions, which appear to be more sensitive than the diaminobenzidine procedure (*10*).

A startling finding was the occasional observation of retrogradely labeled cells within the substantia gelatinosa (Fig. 1, B and C). A total of 50 such cells (1.1 percent of the population of spinothalamic tract cells observed) was found. However, this is an underestimate, since large cells in the region of junction between the SG and the nucleus proprius were excluded from this count. Most (47 SG cells) were contralateral to the thalamic injection site. Eight were relatively large and were not considered typical SG neurons. However, the remainder of the cells were typical of the neuronal population intrinsic to the SG. Although only the proximal dendrites could be visualized, the cells appeared to include both the limitrophe (25 cells) and central cell types (17 cells; see Fig. 2). Thirty-four of the cells were in lamina II and 16 in lamina III.

Although the fraction of the spinothalamic cell population that originates in the SG was small in these experiments, the presence of labeled spinothalamic cells in the SG was a fairly consistent observation in that we observed label in the SG of six of the eight monkeys studied. The negative findings in one animal can be accounted for by the fact that the HRP injection was placed too rostrally to label many spinothalamic cells of any type.

We conclude from our observations, those reported by Giesler *et al.* (*14*), and similar findings in the trigeminal system (*15*) that the mammalian substantia gelatinosa is not a completely closed system but instead includes cells that contribute to long ascending tracts.

W. D. Willis
R. B. Leonard
D. R. Kenshalo, Jr.
Marine Biomedical Institute, Galveston, Texas 77550

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β-Endorphin Is Associated with Overeating in Genetically Obese Mice (*ob/ob*) and Rats (*fa/fa*)

Abstract. *Small doses of the opiate antagonist naloxone selectively abolished overeating in genetically obese mice* (ob/ob) *and rats* (fa/fa). *Elevated concentrations of the naturally occurring opiate β-endorphin were found in the pituitaries of both obese species and in the blood plasma of the obese rats. Brain levels of β-endorphin and Leu-enkephalin were unchanged. These data suggest that excess pituitary β-endorphin may play a role in the development of the overeating and obesity syndrome.*

The genetically obese mouse (*ob/ob*) and the Zucker fatty rat (*fa/fa*) display marked overeating, obesity, hyperinsulinemia, and transient or late-onset hyperglycemia. The syndrome is caused by a single recessive gene, *ob*, located on chromosome 6 in the mouse and a single recessive gene, *fa*, in the rat (*1*). The mechanism for the genetic expression of the syndrome is not known.

One striking difference between obese mice and their lean littermate controls is a 14-fold elevation of pituitary adrenocorticotrophic hormone (ACTH), a polypeptide, in the pituitary of 16-week-old animals (*2*). In addition, there is indirect evidence for elevated corticotrophin-like intermediate-lobe polypeptide (CLIP) (*3*). The ACTH and the CLIP share a common precursor molecule with another polypeptide hormone, β-endorphin. Thus, both β-endorphin and ACTH are

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produced from the cleavage of a single larger precursor (*4*). β-Endorphin and Leu-enkephalin are naturally occurring polypeptides with opiate-like activity that are produced endogenously in all vertebrates studied (*5*). It has been shown that β-endorphin and ACTH are released concomitantly from the pituitary of rats in vivo and also in vitro (*6*). Thus, obese mice and rats would be expected to have higher levels of pituitary β-endorphin as well as of ACTH (*7*). In addition, injection of β-endorphin into the brain (*8*), or subcutaneously injected morphine (*9*) causes an increase in food intake in the rat.

We now report that very small doses of naloxone, an opiate antagonist, selectively abolish overeating in genetically obese mice and rats without affecting the feeding behavior of lean littermate controls (*10, 11*). Moreover, the pituitaries of these obese animals contain twice as much β-endorphin as do pituitaries from their lean littermates (*10*).

In the first experiment seven male and seven female C57BL/6J *ob/ob* mice and equal numbers of lean littermates 2 to 4 months of age were studied. They were kept in individual cages throughout the experiment at 23° ± 2°C on a 12-hour light cycle with lights on from 6:00 a.m. to 6:00 p.m. They were given free access to water; Purina mouse chow was available only between 12 noon and 4:00 p.m. throughout the experiment. The animals were habituated to this feeding schedule for at least 7 days, and on the next 3 days received control injections of 0.9 percent saline 10 minutes before feeding to habituate them to the injection procedure. Each animal then was given subcutaneous injections of naloxone hydrochloride (Endo Laboratories) in 0.9 percent saline at six doses from 0.1 to 5 mg/kg. The order of doses was randomized, and control injections were repeated four times during the experiment. No changes in baseline food intake occurred. The mice were tested with drugs or saline on alternate days. Body weights and 1-hour and 4-hour food intakes were recorded daily. The data were subjected to analysis of variance.

The subjects for experiments 2 and 3 were male C57BL/6J *ob/ob* mice 3 to 5 months of age and their male lean littermates. These animals never received any drug treatment, nor were they deprived of food.

The subjects for experiment 4 were five female Zucker fatty rats 3 to 5 months of age and six female lean littermates (Bird Laboratories). The same 11 animals used for the naloxone experiment were used for the biochemical analysis. Six additional female Zucker fatty rats 2 to 4 months of age that had never received naloxone were added to the biochemical study. In the naloxone experiment with rats the procedures were the same as in the mice experiment with three exceptions: the rats were not deprived of food, a 2-hour feeding measurement period was used, and the drug was injected intraperitoneally.

In the β-endorphin assay, mice and rats were killed by rapid decapitation and their brains were dissected and frozen on Dry Ice. Individual pituitaries were heated in a hot water bath for 15 minutes, homogenized in 2 ml of 2*N* acetic acid with a Brinkmann Polytron, centrifuged at 12,000*g* for 20 minutes, and the supernatant fluid lyophilized to dryness. This extract was resuspended in 10 ml of buffer for radioimmunoassay (RIA) and centrifuged; 50-μl portions were used for assay. In the case of hypothalamus and striatum, which were homogenized in 2 ml of 2*N* acetic acid and finally resuspended in 1.0 ml and 0.7 ml, respectively, 300-μl portions were used for assay. In the β-endorphin RIA, samples were incubated in 0.05*M* phosphate buffer (final volume, 500 μl) at *p*H 7.5 with 0.25 percent bovine serum albumin, 0.5 percent mercaptoethanol, [^{125}I]β-endorphin (5000 count/min), and antiserum diluted 1:2000; the resulting minimum sensitivity was 0.03 pmole (*12*). After overnight incubation at 0°C, bound inodinated β-endorphin was separated from free iodinated β-endorphin by adsorption onto charcoal.

For the Leu-enkephalin assay, whole brains were homogenized in 3.5 ml of 2*N* acetic acid. After the same treatment as above, lyophilized samples were resuspended in 2.0 ml of buffer and 100-μl portions were used for Leu-enkephalin RIA.

Table 1. Opiate peptide content of tissue from obese mice and rats and their lean littermates. Numbers refer to separate experiments. The data were analyzed by Student's *t*-test. NS, not significant.

Tissue measurement	Obese		Lean		*P*
	Mean ± standard deviation	*N*	Mean ± standard deviation	*N*	
	Mice				
	ob/ob		?/+		
Body weight (g)	61.5 ± 3.2	6	33.3 ± 1.8	7	<.001
1) Pituitary weight (mg)	1.23 ± 0.27	6	1.43 ± 0.14	7	NS
Pituitary β-endorphin, RIA (pmoles per milligram of wet tissue)	29.1 ± 9.4	6	18.7 ± 9.4	6	<.02
2) Pituitary weight (mg)	1.63 ± 0.17	11	1.95 ± 0.37	10	<.02
Pituitary β-endorphin, RIA (pmoles per milligram of wet tissue)	27.5 ± 6.3	11	12.7 ± 2.8	10	<.001
Hypothalamus β-endorphin (fmole/mg)	21.6 ± 10.2	7	22.2 ± 5.8	7	NS
Pituitary β-endorphin equivalence, RRA	111.0 ± 31.7	5	54.4 ± 23.2	5	<.02
Whole brain weight (mg)	388.5 ± 15.8	11	437.2 ± 13.7	13	<.001
Whole brain Leu-enkephalin (fmoles per milligram of wet weight)	66.4 ± 13.4	11	60.6 ± 10.0	13	NS
	Rats				
	fa/fa		?/+		
Pituitary weight (mg)	17.0 ± 2.0	6	12.8 ± 2.4	5	<.02
Body weight (g)	706.5 ± 70.3	6	255.4 ± 26.0	5	<.001
Pituitary β-endorphin, RIA	14.7 ± 2.6	6	9.7 ± 1.4	5	<.05
Pituitary weight (mg)	10.4 ± 1.4	6	11.4 ± 1.2	6	NS
Body weight (g)	393.8 ± 56.5	6	234.5 ± 68.7	6	<.01
Pituitary β-endorphin, RIA	17.7 ± 9.0	6	8.7 ± 1.4	6	<.01

The sensitivity of the assay was 0.3 pmole. A modification of the method of Weissman *et al.* (*13*) was used, with a charcoal separation procedure.

In experiment 1, after 1 hour, obese mice ate significantly more food ($P < .01$) than their lean littermate controls (1.54 ± 0.73 g compared to 1.10 ± 0.48 g, respectively; mean ± standard error of mean). A naloxone dose as small as 0.25 mg/kg selectively depressed food intake of the obese animals by 30 percent; resulting consumption was equivalent to that of lean controls (Fig. 1A). When the same data are expressed as percentage of saline controls, dose-dependent decreases in food intake appeared in both genotypes. It should be noted, however, that the suppression of eating in the obese mice was more pronounced at every dose examined between 0.25 and 5 mg/kg (Fig. 1B). The obese mice were ten times more sensitive to the suppressant effect of naloxone than the lean mice. The results of experiment 4 showed a similar effect of naloxone in the rats (Fig. 1, C and D).

A weaker effect could be seen with naloxone after 4 hours of feeding. For example, in obese mice mean consumption of food after the saline injection was 3.5 g; after naloxone at 0.25, 0.5, 1.0, 2.5, and 5.0 mg/kg (subcutaneously administered) mean consumption values were 3.3, 3.0, 3.3, 3.0, and 3.1 g, respectively. In comparison, in lean male littermates mean consumption was 2.3 g after the saline injection and 2.4, 2.2, 2.2, 2.3, and 2.2 g, respectively, after the same series of naloxone injections.

The obese mice and rats received approximately twice the absolute amount of naloxone because of their twofold greater body weight. However, when food intake of obese animals is expressed as percentage of that of saline controls (Fig. 1, B and D), it is possible to compare data for obese animals receiving one dose with those for lean animals receiving twice as much naloxone per kilogram of body weight. In such comparisons, suppression of overeating is greater in the obese mice and rats at every dose of naloxone from 0.50 to 5 mg/kg. These comparisons are helpful in attempting to resolve the critical issue of how to equate doses in genotypes that have different body weight, tissue sizes, metabolism, and so forth.

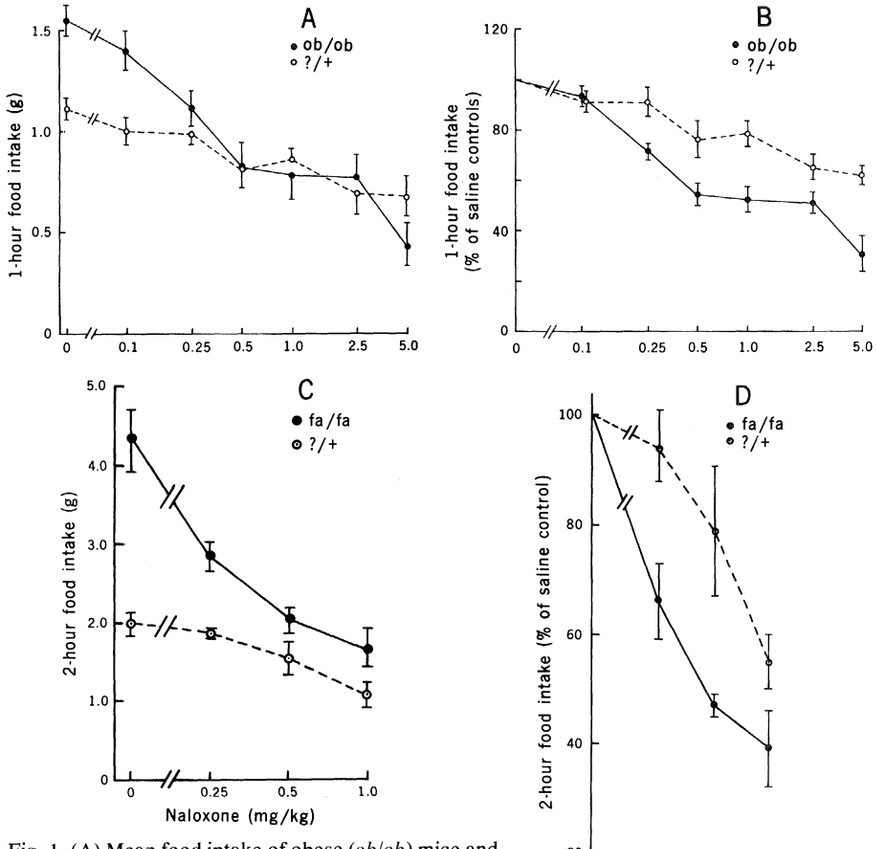

Fig. 1. (A) Mean food intake of obese (*ob/ob*) mice and lean littermate controls (?/+) after various doses of naloxone hydrochloride. The drug was given subcutaneously 10 minutes before feeding. (B) Mean food intake expressed as a percentage of that of saline controls. Subjects were seven male and seven female obese mice *ob/ob* and seven male and seven female lean mice ?/+. There is no significant difference between data for the sexes; thus, they are presented together. The vertical bar length represents ± 1 standard error of the mean. An analysis of variance for the feeding data indicated a significant dose effect ($F = 37.90$; d.f. = 6, 144; $P < .005$) and a significant dose-genotype interaction ($F = 6.88$; d.f. = 6, 144; $P < .01$). (C) Mean food intake of obese (*fa/fa*) rats and lean littermate controls (?/+) after various doses of naloxone hydrochloride. The drug was given intraperitoneally 5 minutes before feeding. (D) Mean food intake expressed as a percentage of that of saline controls. Subjects were 11 female obese Zucker rats (*fa/fa*) and their lean female littermate controls.

The pituitaries from obese mice and rats contain twice as much RIA-assayable β-endorphin as do those of their lean littermates (Table 1). In agreement with previous work (*14*) we found that pituitaries from obese mice weigh 20 percent less than those from controls. We failed to demonstrate any significant difference in whole brain content of enkephalin or in hypothalamic content of β-endorphin (Table 1). These results suggest that regulation of opiate peptide content of brain and pituitary are independently controlled. Moreover, they provide an example of disproportionate ACTH and β-endorphin levels, which suggests that possibilities for uncoupled synthesis or degradation of these peptides exist despite their common genetic product precursor. The radioreceptor assay (RRA) (*6*) of pituitaries (Table 1) revealed a similar twofold increase in β-endorphin equivalent content in the obese mice and rats. The RRA, unlike the RIA, is completely insensitive to the precursor molecule β-lipotrophin. The RRA values are about threefold higher than RIA values, presumably because RRA detects enkephalin and other shorter β-endorphin fragments (such as α-endorphin) which are not detectable by RIA. These results suggest that the difference between obese and lean animals can be attributed to β-endorphin, not β-lipotrophin.

If the excess β-endorphin present in the pituitaries of obese rodents has an endocrinological action, it must be released into the blood. We found a greater than threefold increase in the plasma levels of β-endorphin-like radioimmunoactivity in the obese rat (*fa/fa*) (*15*). This difference suggests that there may be peripheral sites of action involved in the production of obesity by excess levels of β-endorphin.

Continued weight gain in the *ob/ob* mouse is abolished by removal of the pi-

tuitary (*16*). In the present study, the pituitary was the only structure that showed differences in the content of endogenous opiate substances. We assume that the potent effect of naloxone in the abolition of overeating in obese mice and rats is mediated by the antagonism of this material released from the pituitary. If release of excess amounts of β-endorphin from the pituitary is involved in chronic overeating in the obese rodents, a logical target for this action is the opiate receptors in the gastrointestinal tract. Opiate receptors in the ileum, which have been characterized extensively both pharmacologically (*17*) and biochemically (*18*), mediate the pharmacological effects of opiates in suppressing gastrointestinal motility. However, very little is known of their normal role in the gut (*19*).

Our studies suggest that a physiological role may exist for the gastrointestinal opiate receptors in the control of feeding behavior. We cannot rule out the possibility that central opiate receptor sites also participate in the naloxone-induced abolition of overeating. We have shown that a relationship between β-endorphin and obesity occurs in at least two different species and may thus have considerable generality.

β-Endorphin acts on the pancreas to stimulate the release of insulin (*20*). These results are compatible with our findings. Thus, excess pituitary β-endorphin may cause another major physiological system, the endocrine pancreas, to contribute to the overeating and obesity syndrome.

DAVID L. MARGULES*
BEATRIZ MOISSET
Department of Psychology, Temple University, Philadelphia, Pennsylvania 19122
MICHAEL J. LEWIS
Department of Psychology, Tufts University, Medford, Massachusetts 02155
HARUO SHIBUYA
CANDACE B. PERT
Section on Biochemistry and Pharmacology, Biological Psychiatry Branch, National Institute of Mental Health, Bethesda, Maryland 20014

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* Address reprint requests to D.L.M.

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Conservation of Liquid and Solid Quantity by the Chimpanzee

Abstract. *Sarah, an adult "language"-trained chimpanzee, made accurate same-different judgments on quantities of liquid and solid matter and conserved both types of quantity despite a transformation in an irrelevant property (shape). Control tests showed that she judged on the basis of inference rather than perceptual evaluation of the quantities. She failed to make accurate same-different judgments on the basis of number, and she was not tested for conservation of this type of quantity.*

Piaget's classic tests of conservation (*1*) provide dramatic evidence for a developmental change in human cognition. In perhaps the best-known version of the test, the subject is presented with two identical containers filled with equal amounts of liquid and asked to judge the relation between the two quantities. Even the very young child can judge accurately that they are "the same" or "equal." However, transforming an irrelevant property of one quantity (for example, by pouring the contents of one container into another of different proportions, thereby changing the shape of the liquid) reveals a change with age in the subject's response. The young child reports incorrectly that the two containers hold different amounts: one container (typically, the one with the tallest column of fluid) has "more" than the other. The older child continues to judge accurately that the two amounts remain equal—he "conserves" liquid quantity despite a transformation in a vivid, though irrelevant, property.

According to one major theory, the ability to conserve marks the passage from one level of human intelligence (the preoperational stage) to a qualitatively different, more sophisticated level (the concrete operational stage). Conservation demands that the child not be misled by appearance: accurate judgments depend on knowledge or inference. Consequently, much research has been concerned with the conceptual and inferential processes underlying conservation and with finding ways to accelerate the developmental change from one level of intelligence to the next (*2*). However, the limits of the phenomenon remain largely unexplored. Are humans the only animals that ultimately conserve? Evidence for conservation in a nonhuman species could have important implications for our understanding of the cognitive prerequisites for conservation judgments, as well as for comparative theories of intelligence. We report here the results of conservation tests on liquid and solid quantity administered to a nonhuman primate, a chimpanzee.

The subject was Sarah, an African-born female chimpanzee (*Pan troglodytes*), approximately 14 years old. She was obtained by the laboratory when less than 1 year old and taught a simplified language between the ages of 4 years 6 months and 6 years 5 months.

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