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WHY DO WE EAT? A Neural Systems Approach[1]

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ABSTRACT

Neuroregulators found at various brain sites are involved in controlling food intake, a behavior that occurs for many reasons. Different neuroregulators may affect different stimuli that impact eating behavior. For example, neuropeptide Y may initiate feeding for energy needs, opioid peptides may provide the rewarding aspects of eating, and corticotropin releasing factor may affect stress-induced eating. We know that the neural networks regulating feeding also impact other components of energy balance. Neuropeptide Y not only increases eating, it also decreases energy expenditure in brown fat and increases enzymatic activity associated with fat storage in white fat, resulting in a more obese animal. What the sites of action are of these neuroregulators and how they interact with regulators at other sites are of utmost importance. Different regions of the brain, together with the periphery, communicate via signals acting in coordinated fashion, which leads to the final outcome: eating less or more and expending less or more energy.

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INTRODUCTION

The study of neuroregulation of feeding is an exciting challenge. Our task is to integrate observations of complex behaviors with knowledge of specific brain sites and specific brain stimuli. Recent developments in this area have led to significant progress (12). In this review, we attempt to integrate the disparate sources of information by focusing on four themes: that animals and humans eat for a variety of reasons, only some of them having to do with energy deficits; that the neural systems regulating food intake appear to be linked directly to the systems regulating nonshivering thermogenesis and the neural regulation of white fat; that progress in understanding these neural systems will likely come by focusing on the effect of specific neural modulators at specific sites and on the interaction of those site-specific stimuli; and that the neural systems regulating energy intake and expenditure are distributed and interconnected and specifically include, at minimum, the hypothalamus and hindbrain.

WHY DO WE EAT?

Animals and humans eat for a variety of reasons, including energy needs, time of day, social setting, stress, boredom, palatability/reward, and food availability at little or no cost. Historically, studies of energy metabolism have focused on eating initiated by energy deficits and have used various models of food deprivation or restriction. Such models have suggested that a variety of neuroregulatory substances are involved in the regulation of food intake. More recently, studies conducted with palatable foods indicate that neuroregulators also affect intake stimulated by the taste/reward from foods.

Many neuroregulators increase animal feeding, including neuropeptide Y (NPY), opioids, galanin, norepinephrine, and benzodiazepines (67, 70, 71, 95). Similarly, many neuroregulators decrease feeding, including corticotropin releasing hormone. These agents produce their food-intake effects after injection into the cerebroventricles or into specific brain nuclei. To focus our discussion, we primarily consider NPY and opioids, which provide examples of how neuroregulators may be involved in two different aspects of energy metabolism.

Energy Deficit–Induced Feeding

Substances critical to feeding in response to an energy deficit demonstrate increased signaling, manifested as increased amounts of the neuromodulator and/or its mRNA following food deprivation/restriction. Conversely, levels of the neuroregulator and/or its mRNA decrease after restoration of food or during caloric excess. Neuromodulator release from neurons, when it can be measured, changes reciprocally with energy balance if the agent in question is to participate in the response to altered energy status.

NEUROPEPTIDE Y NPY, a potent orexigenic agent, is synthesized and active in a variety of neural circuits (27, 76, 125). An energy metabolism pathway of interest is the synthesis of NPY in the arcuate nucleus of the hypothalamus, with axonal transport to the paraventricular nucleus of the hypothalamus (PVN) (11). This projection is of particular importance, because the arcuate appears to have the greatest density of cell bodies expressing NPY within the hypothalamus. There is strong evidence for primary projection of these cell bodies to the PVN, and in the PVN concentrations of NPY, peptides are particularly high. NPY receptors are also found within the PVN, and so, it appears that the essential components are present anatomically. Furthermore, there is considerable evidence for changes both in NPY messenger RNA expression in the arcuate and in the NPY peptide itself in the PVN in response to experimentally produced changes in energy balance. Messenger RNA levels of NPY in the arcuate nucleus have been shown by many groups to increase following food deprivation or food restriction (16, 26, 30).

Chronic food restriction increases NPY gene expression more potently than does short-term (48 h) food deprivation (16). Food restriction also increases peptide levels of NPY in the PVN (108). In an in vitro study, Dube et al found that NPY release was elevated in microdissected PVN of food-deprived rats (33). Other manipulations that result in an energy deficit also alter gene expression or peptide levels of NPY. For example, rats made diabetic by injection of streptozotocin have increased NPY mRNA levels in the arcuate nucleus and increased peptide levels in the PVN (136). The diabetic rats are hyperphagic, but they gain body weight at a much slower rate than control animals do. In the lactating rat, NPY levels are increased in the arcuate nucleus-median eminence complex, PVN, ventromedial nucleus, and dorsomedial nucleus (87). NPY mRNA levels are increased in the arcuate nucleus of lactating rats (121). Intense voluntary running in rats increases NPY peptide levels in arcuate, dorsomedial, medial preoptic, and lateral hypothalamic areas in a fashion similar to that caused by food restriction (82), presumably by creating a relative energy deficit. Schedule feeding is another means of ensuring high rates of food intake in a short time period. Using push-pull methodology, Kalra and colleagues reported that NPY is released from the PVN in schedule-fed rats and that peak levels occurred prior to meals (54).

Several behavioral studies have indicated that NPY robustly increases motivation to eat. Rats injected intracerebroventricularly with NPY are highly motivated to press levers for food (51). We studied the effect of NPY on pressing levers for food when the number of presses required to obtain food was progressively increased (52). The break point—the number of responses needed to obtain the last reinforcer—was increased with NPY to a level comparable to 36–48 h of food deprivation. These studies suggest that both NPY-induced and

deprivation-induced feeding stimulate an equivalent motivation to eat. Drug discrimination has also been applied to evaluate whether or not NPY- and deprivation-induced feeding are similar. Two laboratories trained rats to discriminate between a central injection of NPY and a saline injection (53, 112). In both studies, food-deprived rats (24 or 48 h) failed to select the NPY lever, suggesting that deprivation "feels" different from NPY. However, it should be noted that 24- or 48-h food deprivation initiates interoceptive cues over a time period different from that required for interoceptive cues of a bolus peptide injection. A better test might be one that trains rats to discriminate between satiation and hunger, followed by testing with NPY.

Seeley et al studied the effect of NPY and 24-h food deprivation on intraoral intake in rats (113). In this paradigm, a rat is fitted with an intraoral cannula in which a 0.1 M sucrose solution is infused, forcing the rat to either swallow the solution or let it drip out of its mouth. A computer-driven pump stops the infusion when the animals are not ingesting the solution. NPY (3–30 μg intracerebroventricularly) failed to change intraoral intake compared with saline controls, and food deprivation increased intake almost twofold. These data suggest that NPY does not alter the consummatory phase of ingestion and that it does not mimic food deprivation-induced changes in intraoral consumption.

We also compared behavioral effects of NPY to food deprivation by directly observing the behavior of rats once per minute over a two-hour period (74). We found that while 24-h food deprivation and intraventricular injection of NPY increased feeding similarly, the behavior of the rats differed between the two treatments. Animals given NPY moved more and groomed less than did the food-deprived rats. When we removed food from the cage, the NPY-injected rats moved around the cage much more than the food-deprived rats did, which suggests that NPY affects exploratory behavior (perhaps searching for food). A longer deprivation (48 h) for food-deprived rats increased their movement, but not to the degree that was seen after administration of NPY.

Lynch et al evaluated the effect of NPY and food deprivation on the pattern of intake of sweetened, condensed milk (86). NPY increased both total ingestion time and total volume consumed during the 1-h test. While NPY increased the number of licking bouts and decreased the pauses between bouts, it decreased the size of each bout, the length of the bout, and the rate of licking during a bout. This pattern did not resemble the one seen with food deprivation. Analysis suggests that NPY decreased satiety without affecting palatability. Food deprivation increased the initial rate of licking, without altering the rate of satiation, and increased the individual bout size and duration, without altering total number of bouts. Thus, the Lynch et al study suggests that NPY does not enhance orosensory stimulation but does slow the satiation rate, which is a pattern different from food deprivation.

ENDOGENOUS OPIOIDS In 1963, Martin et al (89) reported that morphine-addicted rats ate large amounts of food after daily injection of morphine. Grandison & Guidotti (40) noted that injection of ß-endorphin into the ventromedial region of the hypothalamus increased feeding in rats. These observations, combined with the finding that naloxone decreased food and water intake in rats (77), initiated the notion that opioids/opiates might be involved in the regulation of food intake. From that start, three families of opioid peptides and three classes of receptors (recently a fourth receptor, ORL_1, has been cloned) have been identified (2, 88, 103). Mapping studies have indicated that a wide variety of brain nuclei contain both opioid receptors and opioid peptides (88). This has resulted in a flurry of scientific activity attempting to identify which opioid receptor subtypes are involved in consummatory behaviors. In addition, much work has focused on which aspects of feeding behavior are influenced by opioids.

Unlike NPY, endogenous opioids of the mu, delta, kappa, and ORL_1 receptors do not stimulate feeding in a potent fashion, which suggests that opioids may not be directly involved in initiation of feeding. If this were the case, one would not expect opioid gene expression to be increased in response to an energy need. Brady et al found that food restriction resulted in a decrease, rather than an increase, in mRNA levels of proopiomelanocortin (POMC) in the arcuate nucleus (16). We noted that food deprivation and food restriction decreased mRNA levels of POMC, as well as enkephalin and dynorphin mRNA levels, in the arcuate nucleus (57). In contrast, hyperphagia stimulated by palatability resulted in increased mRNA levels of dynorphin in the arcuate nucleus (132). Dynorphin A levels in the PVN were also elevated in these hyperphagic rats. Preliminary data from our laboratory indicate that gene expression of opioid peptides is decreased in the arcuate nucleus of hyperphagic lactating rats. Thus, in contrast to NPY, opioid gene expression appears to be decreased in rats experiencing an energy deficit, which suggests that opioid peptides do not directly participate in feeding initiated by an energy deficit.

Carr et al conducted a series of studies using autoradiography to evaluate opioid binding in food-restricted rats (22, 137). They studied more than 50 brain regions and, after food restriction, found changes in only six areas. Mu opioid binding decreased in the basolateral-basomedial amygdala, parabrachial nucleus, and habenula. Kappa opioid binding decreased in the habenula but increased in the bed nucleus of the stria terminalis, ventral pallidum, medial preoptic area, and parabrachial nucleus. Berman et al also measured prodynorphin-derived peptides in food-restricted (7) and diabetic rats (8). They found that dynorphin A_{1-17} levels increased in dorsomedial, ventromedial, and medial preoptic hypothalamic areas and decreased in the central amygdala. Levels of dynorphin A_{1-17} increased in the ventromedial nucleus and dorsomedial nucleus

of diabetic rats. The data from Carr et al emphasize the importance of examining a variety of brain nuclei. For example, they noted an increase in dynorphin $A_{1–17}$ in several hypothalamic regions of food-restricted rats but a decrease in the amygdala. Both of these brain nuclei are important regions in the regulation of food intake.

Studies with a host of selective opioid receptor antagonists have been conducted in rats stimulated to eat due to an energy deficit (14). Mu-selective opioid receptor antagonists potently decrease (42–50%) deprivation-induced food intake compared with delta (no effect) and kappa (28%) antagonists (3, 4, 14, 72, 73). While naloxone decreases short-term food deprivation, its effects on chronic food restriction or schedule feeding are fairly weak (78, 81, 134). Also, the effectiveness of opioid antagonists on feeding stimulated by energy deficits seems to depend on the type of food presented (134). Opioid antagonists also decrease glucoprivic feeding following injection of insulin or 2-deoxy-D-glucose and have a greater effect on the latter (75, 83, 100).

Egawa et al reported that ß-endorphin decreases sympathetic nerve activity in brown adipose tissue, which suggests a potential role for opioids in thermogenesis (36). Opioids also have other effects on metabolism. For example, morphine and selective opioid agonists result in hyperglycemia (43). In pigs, intravenous morphine increased oxygen consumption, carbon dioxide production, and plasma concentrations of lactate, glucose, epinephrine, and norepinephrine (15).

Hedonic-Induced Feeding

In a society like ours, much overeating occurs as a result of readily available, inexpensive, and good-tasting foods. Many of these foods contain large amounts of fat and sugars and are, therefore, energy dense. Aside from taste, qualities such as texture and smell also contribute to overconsumption of food. A variety of neuroregulatory systems are involved in taste perception, palatability, or hedonic values of foods, such as smell and texture. Once again, we focus on the possible involvement of NPY and opioids in hedonic-induced feeding.

NEUROPEPTIDE Y While NPY appears to be primarily involved in eating stimulated by energy needs, there is some evidence that NPYergic neurons affect reward-driven feeding. Lynch et al found that intraventricular injection of NPY increased intake of sucrose and consumption of saccharin in satiated rats more effectively than the intake of saline solutions did (85). In addition, palatable sucrose solutions flavored with orange or black cherry Kool-Aid® were selectively associated with NPY injection during single-bottle training sessions. Subsequent two-bottle preference tests demonstrated a shift in preference toward the flavor paired with NPY during training. NPY seems to stimulate ingestion of

sweet solutions and may potentiate sweet taste preference by an associative mechanism. In contrast, Sipols et al noted that central administration of NPY in doses that stimulated eating caused formation of conditioned flavor aversions (119), although the study differed because the Kool-Aid® was unsweetened and food was available during conditioning. It is possible that NPY stimulated an excess amount of food intake during conditioning, which resulted in subsequent distress or nausea.

A variety of studies have indicated that when allowed to select from three macronutrient diets containing carbohydrate, protein, or fat, rats injected centrally with NPY preferentially chose carbohydrate (67, 98, 124, 127). While this seems to be the case, Welch et al found that an animal's preexisting preference for a macronutrient plays an important role in this NPY-induced carbohydrate preference (131). An animal that preferred carbohydrate was more likely to choose carbohydrate than one that preferred fat. This effect was also noted when rats were given a choice between a high-carbohydrate and high-fat diet. Our preliminary data indicate that the carbohydrate source (starch, polycose, sucrose) alters a rat's food preference, and NPY increases preferred carbohydrate intake.

ENDOGENOUS OPIOIDS The role of opioids in palatability/reward (28, 104) has been an attractive hypothesis. Blockade of opioid receptors by naloxone injection reduces intake of saccharin, sucrose, and saline solutions more effectively than water or quinine solutions (79). Yirmiya et al (138) found that opioid receptor-deficient mice (CXBK) had lower saccharin preference than control mice did. Rockwood & Reid (105) noted that naloxone reduced intake of a 10% sucrose solution in food-deprived and non–food-deprived sham-drinking rats, which indicates that naloxone's antidipsogenic actions were not due to feedback from postabsorptional signals. The intake pattern of sham-fed animals given a 10% sucrose solution and injected with naloxone was identical to that of rats ingesting a 5% sucrose solution without administration of naloxone, which suggests a change in the perceived quality of the sucrose solution (62). Measuring the hedonic properties of taste, Parker et al (101) found that naltrexone reduced the positive hedonic properties of sucrose solutions in rats. Lynch (84) reported that very low doses of naloxone (0.1 mg/kg) reduced intake of saccharin solutions in nondeprived rats. Lynch also found that preference acquisition for saccharin solution was blocked by daily pretest naloxone administration. In six-day-old rats, Shide & Blass (116) showed that orange odor preference development, after pairing it with intraoral sucrose infusion, was blocked by naloxone administration.

Naloxone seemed to block that portion of feeding driven by sweet taste in food-restricted rats (81). Using sweet chow and various deprivation states to evaluate the interaction of reward and deprivation, we found that naloxone's

anorectic potency was dependent on the type of chow presented to the rats and on the deprivation schedule utilized to stimulate food intake. In rats deprived for 24 and 48 h, naloxone decreased intake of normal rat chow at doses ranging from 0.3 to 3.0 mg/kg. In chronically deprived rats (80% of normal body weight), these doses of naloxone failed to decrease intake of normal chow. In both acute and chronic food-deprived groups, rats eating sweet chow ate more when they were energy deprived and were more sensitive to naloxone-induced changes in food intake than were rats eating normal chow. Thus, naloxone decreased the intake of sweet chow more effectively than of normal chow, even when rats were chronically food deprived. In satiated rats, an extremely low dose of naloxone (0.03 mg/kg) decreased intake of sweet chow by almost 50%. These data suggest that, in hungry rats, naloxone primarily blocked the portion of feeding that was driven by sweet taste.

To test whether the source of the carbohydrate (CHO) given to rats affected naloxone's anorectic effects, we provided rats with diets that differed only in the type of CHO included—cornstarch, sucrose, or polycose (134). These diets were offered both to rats with free access to food and to rats that were food restricted. We assumed that food intake was driven primarily by both energy needs and palatability in rats fed ad libitum; in food-restricted rats, we expected intake to be driven by energy needs, with a relatively small palatability component in the preferred sucrose and polycose diet groups. In rats fed ad libitum, naloxone reduced nocturnal intake of all three diets at doses of 0.3, 1.0, and 3.0 mg/kg, respectively. Naloxone failed to alter intake of the cornstarch diet in rats chronically food restricted. However, naloxone decreased intake of the sucrose diet in food-restricted rats at doses of 0.3, 1.0, and 3.0 mg/kg and decreased intake of the polycose diet at the 3.0 mg/kg dose. Once again, naloxone had its major impact on food intake induced by a factor other than energy needs.

Recently, we studied the effect of naloxone on NPY-induced and deprivation-induced food intake in rats able to choose between a high-CHO and a high-fat diet (39). At doses as high as 3.0 mg/kg, naloxone failed to decrease intake of the less-preferred diet in the food-deprived rats. In contrast, naloxone was able to decrease intake of the preferred diet by 22% in these rats, at doses as low as 0.01 mg/kg. Similar effects were observed when rats were induced to eat by intraventricular injection of NPY. In this case, 0.01 mg of naloxone/kg decreased intake of the preferred diet by 36%. The above studies substantiate the idea that endogenous opioids are involved in the rewarding properties rather than the energy content of foods. The finding that extremely low doses of peripheral naloxone decreased intake of preferred food suggests that previous studies using doses of naloxone ranging from 3 to 10 mg/kg may not have involved physiological processes and, therefore, represented pharmacological effects of this opioid antagonist.

Using electrical brain stimulation, Carr & Simon (21, 23–25) demonstrated a role for opioids in palatability-related feeding. Stimulation within the lateral hypothalamic medial forebrain bundle can elicit self-induced feeding. Peripheral naloxone injection increases the threshold necessary to produce self-induced feeding (23, 25), a shift that is similar to decreased food palatability. Opioid blockade appears to alter meal maintenance without affecting initiation, which is a pattern consistent with the notion that opioids affect palatability/reward rather than affecting hunger directly (59–61).

Ingestion of palatable solutions also alters opioid peptides and/or receptors. Dum et al (34) reported that consumption of chocolate milk or candy decreases [^{3}H]etorphine binding and ß-dynorphin content in the hypothalamus of rats, perhaps owing to a release of ß-endorphin. ß-Endorphin–like immunoreactivity was elevated in the pituitary of rats made obese by prolonged feeding of palatable foods (42). Prodynorphin mRNA levels in the PVN and the supraoptic nucleus were positively correlated with consumption of a high-fat diet (17). We found that mRNA levels of prodynorphin were elevated in rats exposed to a high-fat/high-sucrose diet (133). Chronic ingestion of a glucose-saccharin solution, which might release endogenous opioids, decreased morphine-induced analgesia (6). Oral infusion of sucrose in rat pups increased paw-lift latencies in the hot-plate analgesia test and decreased distress vocalization, and both changes were naltrexone-reversible (13).

Welch et al evaluated the effect of a highly palatable diet on hypothalamic peptide and mRNA levels of opioids (133). They fed rats either a cornstarch-based diet (CHO) or a high-fat diet containing sucrose (Fat/Sucrose). Rats received either CHO ad libitum, Fat/Sucrose ad libitum, Fat/Sucrose pair-fed to the caloric intake of CHO, or Fat/Sucrose at 60% of ad libitum Fat/Sucrose intake. The rats eating the Fat/Sucrose diet ad libitum consumed more calories and gained more weight than did the other groups. Relative to CHO, ad libitum intake of Fat/Sucrose elevated prodynorphin (proDYN) mRNA levels in the arcuate and dynorphin $A_{(1–17)}$ levels in the PVN, but it did not alter arcuate mRNA levels of proenkephalin (proENK) or POMC or PVN peptide levels of Met-enkephalin or ß-endorphin. Pair-feeding the Fat/Sucrose diet to the level of intake of the CHO diet resulted in levels of proDYN and dynorphin $A_{(1–17)}$ similar to the two-diet groups. Pair-feeding Fat/Sucrose reduced mRNA levels of all opioids and dynorphin $A_{(1–17)}$ levels, relative to ad libitum feeding of Fat/Sucrose. Met-enkephalin and ß-endorphin were not affected by dietary treatment. Feeding Fat/Sucrose at 60% of ad libitum intake resulted in opioid mRNA and dynorphin $A_{(1–17)}$ levels similar to those observed in the CHO group. Thus, hypothalamic dynorphin $A_{(1–17)}$ and proDYN mRNA levels were stimulated by a highly palatable diet rich in fat and sucrose. The increased synthesis may be due in part to over-consumption of calories caused by palatability.

Operant studies were conducted to evaluate whether feeding increased by opioid agonists or feeding decreased by an antagonist affected initiation or maintenance of the meal. The idea is that the time before feeding begins should be shortened by hunger (decreased latency). In contrast, intake provoked by palatability would require direct experience of the food and, therefore, would not be associated with decreased time to begin eating. Rather, rewarding foods would be anticipated to promote longer meals as the animal seeks greater exposure to the rewarding stimulus. To test this hypothesis, Rudski et al (107) injected naloxone into rats responding to a schedule designed to differentiate between feeding latency and meal maintenance. Naloxone did not increase time prior to eating (latency). Naloxone suppressed the maintenance phases within the first 10 min of the sessions. Naloxone's suppression of subsequent intake and the lowest effective dose were inversely related to deprivation level. These results agree with the studies summarized above indicating that naloxone does not reduce feeding in deprived rats except at very high doses. Rudski et al also studied the effects of the mixed opioid agonist/antagonist, butorphanol, on food-reinforced responses in satiated rats. Vehicle administration was associated with consumption of relatively large quantities of food only within the first 10 min of receiving the first pellet, but butorphanol was associated with continued feeding as the session progressed. Buprenorphine, another mixed opioid agonist/antagonist, also increased free feeding and operant responses in satiated rats (107). Buprenorphine failed to affect the task associated with meal initiation, but it increased the total amount of time spent on continuing the meal. These data suggest that opioids play a role in feeding maintenance, rather than initiation, which is compatible with the hypothesis that opioids affect something other than energy-deficit– induced feeding.

In humans, naltrexone was found to decrease the pleasantness of a sucrose solution (9, 37), and naloxone reduced intake of sweet high-fat food in binge eaters but not control subjects (32). To test whether or not opioids directly alter the taste of a preferred substance, O'Hare et al employed drug discrimination methodology in which two groups of rats were trained to discriminate either a 10% or a 5% sucrose solution from water (99). These mildly food-deprived rats (95% of free-feeding weight) were trained to press the appropriate lever in a two-lever operant chamber following a sampling of the test stimulus; successful responses were reinforced by food. After random exposure of both training groups to reduced sucrose concentrations, a sucrose concentration gradient was established. Data from saline-treated rats were then compared with those obtained following random i.p. naloxone administration. Rats trained on 5% or 10% sucrose distinguished lower concentration of sucrose extremely well. Compared with vehicle-injected control animals, naloxone failed to alter ability to discriminate. Thus, it appears that naloxone does not affect the ability to taste a substance, but it may alter the rewarding properties of that substance.

Stress-Induced Feeding

The concept that organisms respond to stress in a unified fashion was first championed by Hans Selye. The hypothesis called for a system in which one regulator would initiate events leading to a general physiological response. A host of stressors—including family separation, child abuse, and parental neglect—can lead children to compulsive intake of clay, soil, paint chips, and grass (109, 118). Patients with posttraumatic stress disorder suffer from anxiety, depression, drug abuse, and eating disorders (92). Stress-related oral behaviors include bruxism in anxious children; hyperphagia in women prisoners; and increased tongue, lip, and finger biting in Lesch-Nyan patients during stressful periods (66, 111, 114). Kaye et al have reported that bulimic behavior can relieve stress (55). Greenberg found that life stress, depression, dietary restraint, and binge eating scores were deviant in bulimic women compared with those of control subjects (41). Animals also seem to respond to stress by altering their eating behavior. Loud sounds, isolation, confinement, electric shock, and tail pinch all lead to changes in feeding in rats (70).

CORTICOTROPIN RELEASING HORMONE Many neuroactive substances have been implicated in stress responses. Of particular interest is corticotropin releasing hormone (CRH), putatively the substance first envisioned by Hans Selye in his concept of the stress response. CRH was identified in 1981 as a hypothalamic releasing factor that stimulates expression of proopiomelanocortin (POMC) (122, 129). We now know that CRH affects endocrine, immune, cardiovascular, and gastrointestinal functions and influences behaviors such as sexual activity, locomotion, grooming, and feeding (19, 97). Also, the injection of CRH antisense and the functional impairment of hypothalamic CRH neurons with immunotargeted toxins both increase food intake (50, 90). CRH decreases feeding stimulated by food deprivation or by administration of muscimol, norepinephrine, dynorphin, insulin, and NPY (80). The decrease in eating and increase in grooming observed after CRH administration is seen in hypophysectomized and adrenalectomized rats (97). This suggests that CRH's effect on feeding is independent of ACTH and cortisol, substances generally assumed to be involved in stress. Krahn et al found that CRH decreased feeding while increasing grooming in rats after it was injected into the PVN but not after injection into the striatum, globus pallidus, ventromedial hypothalamus, or lateral hypothalamus (65). Ganglionic blocking agents attenuated CRH-induced grooming in rats, which suggests involvement of the sympathetic nervous system in some of CRH behavioral effects (18).

The effects of CRH on feeding may be directly related to stress-related changes in feeding. For example, Krahn et al found that alpha-helical CRH-(9-41), a CRH antagonist, reversed the decrease in feeding that was seen after physical restraint (64). CRH injection and physical restraint both result

in decreased gastric acid secretion, gastric emptying, and small bowel transit time. These effects of restraint could be reversed with administration of alpha-helical CRH-(9-41). Shibasaki and colleagues also found that this CRH antagonist decreased the effect of restraint on food intake (115). Heinrichs & Koob studied the effect of CRH in nutritionally stressed rats that were given a protein-deficient diet (49). The rats with a deficient diet demonstrated an increased preference for unfamiliar foods as compared with nutritionally replete rats. CRH and physical restraint decreased consumption of the novel food in the deficient rats without affecting their intake of a familiar food. There was no effect of CRH on consumption of either diet by the replete control group. Also, alpha-helical CRH-(9-41) reversed the anorexia produced by CRH and physical restraint.

Urocortin, a new member of the CRH family, decreases free-feeding and deprivation-induced feeding in rats at much lower doses than those observed with CRH (123). Urocortin decreases feeding without producing either an anxiogenic-like behavior or the conditioned place aversion that occurs after CRH administration. Urocortin decreases meal size without decreasing the frequency of meals, a pattern noted with other anorectic substances.

The effect of stress on neuropeptide gene expression has not been examined systematically. One problem is defining rat stress. Chronic food restriction might be a stressful event, but Brady et al found a decrease in gene expression of CRH in the arcuate nucleus after food restriction (16).

ENDOGENOUS OPIOIDS Release of CRH after stress results in release of ß-endorphin, a part of the POMC molecule. The blockade of opioid receptors decreases the levels of stress hormones such as prolactin as well as growth hormone (94). Exercise stress alters opioid levels and appears to interact with feeding behavior (38, 45). For example, naloxone, an opioid antagonist, blocks the effect of acute swim stress on rat feeding (130). Cold swim stress also increased ß-endorphin and dynorphin levels in the hypothalamus. Tail pinch–induced feeding, a model of stress-induced feeding, is decreased by naloxone administration (96). Administration of naloxone to chronically tail-pinched rats resulted in a withdrawal-like response, including "wet dog" shakes. This suggests that tail pinch– induced feeding may result from a stress-related release of opioids, which in turn influences feeding.

Kehoe & Blass found that morphine administration decreased distress vocalization, which occurs in infant rats when they are separated from their mothers (56). Intraoral administration of milk, fat, and Polycose® to isolated rats also decreased distress vocalization. All these effects were blocked with pretreatment of naltrexone, an opioid antagonist. Isolation and drinking milk also resulted in analgesia as measured by an increase in paw-lift latency on a hot

plate. This analgesic and calming effect of milk ingestion in rat pups seems to be related to opioidergic pathways.

NEUROPEPTIDE Y While a variety of reports suggest that NPY is an endogenous anxiolytic agent, the effect of NPY on stress-induced changes in feeding has not been studied in detail (46, 47). Several studies demonstrate an interaction between NPY and CRH. For example, centrally administered CRH attenuates NPY-induced feeding, whereas pretreatment with a CRH antagonist enhances NPY-induced feeding (48, 98). Also, inactivation of CRH neurons in the paraventricular nucleus using ricin A enhanced feeding stimulated by paraventricularly administered NPY (90). Many reports indicate a relation between NPY and steroid hormones, particularly glucocorticoids. Leibowitz et al have found that NPY increases release of corticosterone, which suggests a relationship between NPY, CRH, and stress (68). Also, NPY gene expression is enhanced by corticosterone administration, and corticosterone potentiates NPY transport, release, and receptor function (1, 29, 31, 68, 128, 135).

INTEGRATED ENERGY MANAGEMENT SYSTEM

The significance of the neural networks that regulate feeding is now greatly enhanced by data indicating that these same networks regulate additional components of energy balance. It has long been known that energy expenditure, at least that part of energy expenditure known as nonshivering thermogenesis, is a regulated process and that it plays an important regulatory role in the nervous system. For example, data show that energy expenditure in animals can be either up-regulated in response to chronic over-nutrition or down-regulated by chronic undernutrition (106). Similarly, human studies show energy expenditure responses to nutritional status (91, 117).

Experience with NPY is a good example of this general phenomenon. NPY was originally thought to participate in energy management because it was observed that centrally administered NPY was a potent stimulator of feeding. This feeding stimulation appears to take place at several neural sites, but most evidence indicates that the paraventricular nucleus is the critical site for NPY effects. Studies in the past several years have shown that NPY not only potently activates food intake, it simultaneously suppresses energy expenditure in brown fat and increases enzymatic activity associated with fat storage in white fat (10, 11). Data indicate that NPY central stimulation promotes energy storage in fat at a rate greater than can be accounted for by enhanced food intake, which is consistent with the observed metabolic effects on brown and white fat activity. In addition, data indicate that central NPY stimulation can suppress sympathetic nervous system activity in the innervation of brown fat (35). These

data further show that the effects on brown and white fat take place whether or not the food intake is allowed to occur, which indicates a direct neural effect independent of the food. NPY injected intracerebroventricularly decreases insulin-stimulated glucose uptake in skeletal muscle while it increases insulin-mediated glucose uptake in adipose tissue (139). Zarjevski et al also noted that hepatic and adipose lipogenesis occurred after NPY injection (139). Thus, NPY may be a regulator of a central energy metabolism circuit.

These observations indicate innervation of brown and white fat. In the case of brown fat, the evidence for innervation is well developed through many studies exploring the role of innervation in brown adipose tissue. It is further known that the sympathetic nervous system is the principal system involved, which is consistent with the other observations in this area. What has been less clear is the potential for innervation of white fat. Circumstantial evidence implicating the potential neural regulation of white fat has existed for a long time. However, demonstrating the connections histologically has been challenging. In the past few years, critical evidence has shown that white adipose tissue is innervated, and that the neural connections to white fat trace back to sites within the hindbrain known to be involved in autonomic regulation (5).

Another possible mechanism for NPY action might be through changes in insulin status. Central injection of NPY has been reported to increase serum insulin 30–120 min after injection (110). We have not noted a long-term (24 h) effect of NPY on insulin levels. Brown and white fat metabolism could also be affected by NPY-induced changes in ACTH and corticosteroid levels. Unfortunately, the effects of NPY on the hypothalamic-pituitary-adrenal axis are unclear, since some investigators report NPY-induced changes in corticosteroids, while others note no changes.

There are many other examples of coordinated regulation of different energy balance effectors by neural regulatory networks. A prominent recent example is the peripherally synthesized adipose hormone called leptin. Leptin administered to both mice and rats results in a dramatic decrease in food intake but also in increased energy expenditure, nonshivering thermogenesis, and—in some cases—body temperature (20, 44, 102). There are some indications that leptin may work, in part, through NPY pathways in the arcuate and PVN (126). The opposite appears to occur for the central neuropeptide, CRH, which, in addition to its role in regulation of the hypothalamic-pituitary-gonadal axis, appears to have a role in regulation of food intake. Administration of CRH centrally has also been shown to enhance nonshivering thermogenesis in brown adipose tissue. Parallel cases occur for the central anorectic stimuli of amphetamines and serotonin. Drugs of these types are used clinically for management of appetite, and there are animal studies associating enhanced energy expenditure with these stimuli.

These examples and many others illustrate a consistent property of the regulatory system involving food intake: Food intake is regulated as only one of the variables controlled by a neural system that regulates energy balance. There must be coordinating elements in that neural system because these elements apply to several different energy balance effectors and appear responsive to many different stimuli. At the same time, there must be specific elements that ultimately produce a behavior, such as feeding, that are distinct from pathways regulating sympathetic innervation to brown fat or white fat. Similarly, neural differentiation likely occurs at the point of input of signals that are being processed; for example, leptin is one type of stimulus from the periphery, but the desire to eat in response to the recognition of palatable food may originate at a different neural site. An ongoing challenge, then, is to identify the components of the neural systems that regulate energy balance and correspond with these different functions. It appears, in fact, that neural systems can be considered analogous to the principal components of a computer: There are inputs, processing units, and output regulating sites—all within the brain.

SITE- AND STIMULUS-SPECIFIC REGULATION

Within the literature on feeding and obesity, neuropeptide Y is commonly thought of as a feeding stimulatory peptide. We know, however, that NPY is one of the most abundant and widely distributed neuromodulators within the neuraxis and that NPY has a wide variety of functions within the brain. Even within the hypothalamus, NPY regulates hormonal components that relate to reproduction and, probably, those that relate to blood pressure, in addition to its effects on energy metabolism. As a consequence, it is ever more important to focus on the site- and stimulus-specific description of a particular stimulus, such as NPY; accordingly, we focus on the arcuate to PVN circuit. There are persistent questions about the meaning of NPY in the PVN itself. Since NPY measurements, as they are commonly performed, do not distinguish between presynaptic, intrasynaptic, and postsynaptic location for NPY, it is sometimes difficult to ascribe a specific meaning to an increase or decrease in NPY density within the PVN. A better approach is assessment of the NPY release at a specific site, and the limited data that results from using this approach confirm that, at least in the case of deprivation and/or scheduled feeding, NPY within the PVN functions as an indicator of increased activity within this pathway. It is also recognized that NPY has effects on feeding at several other brain sites. Notably these sites include the perifornical area of the hypothalamus, which is near the PVN but, nonetheless, a different site. Several other sites have been shown to be sensitive to NPY with respect to increased food intake, including the ventromedial area of the hypothalamus and certain sites within the brainstem.

There are parallels to NPY in other neuromodulators and their site-specific influence on feeding. Norepinephrine has feeding stimulatory effects at various brain sites, notably the paraventricular nucleus within the hypothalamus (93). However, norepinephrine, like NPY, is a very widely distributed neuromodulator that performs a great many actions within the brain and peripheral nervous system (69). Cholecystokinin (CCK) is generally recognized as a satiety influence (120). CCK is released by the gut peripherally and appears primarily to act peripherally at sites relating to the vagus nerve. Nonetheless, CCK has a variety of physiologic effects that are entirely unrelated to satiety. The CCK story is further complicated by the fact that CCK can function as a neuromodulator within the central nervous system, and it appears that CCK can have anorectic effects at some brain sites. CRH appears to have important effects on feeding that are separate from its ACTH regulatory activities noted above. Again, the site is of particular importance because the action of CRH in the median eminence will be expected to affect ACTH primarily. On the other hand, CRH in the PVN has important effects on feeding and ACTH regulation. It is difficult to cite these neuromodulators as feeding-regulatory influences unless one is specific about the sites and conditions in which they act.

DISTRIBUTED INTERCONNECTED NETWORK

The characteristics of the food intake regulatory system cited above included the disparate reasons why animals eat, the connection between regulation of food intake and energy expenditure, and the sites of neuromodulator action. The effect of NPY on food intake can be blocked by peripheral administration of the opioid antagonist naloxone or a similar opioid antagonist species. The simplest way to understand this blockade effect is by assuming that an opioid blockade would act, somehow, in the paraventricular nucleus to inhibit the NPY effect on target neurons. In studies in our laboratory, it became clear that naloxone, given at the PVN site, could not effectively block NPY effects. A search for potential neural sites for opioid blockade led to the recognition that naloxone or naltrexone administered into the nucleus of the solitary tract could completely eliminate the effect on output variables, such as food intake or energy expenditure, which would otherwise be produced by NPY stimulation of the paraventricular nucleus (63). These data suggested that there must be a functional projection from paraventricular nucleus to nucleus of the solitary tract, and indeed, anatomical evidence to support this connection was available (58).

Several lines of evidence indicate that there are functional connections between the hindbrain and potential processing sites in the hypothalamus,

including the paraventricular nucleus. A well-defined set of neuroanatomical projections linking the NTS, the parabrachial nucleus, and other hindbrain sites with the hypothalamus has been identified. Further, it is possible to regulate food intake by manipulations within the hindbrain without directly affecting otherwise important sites within the hypothalamus. Similarly, it is possible to affect food intake in the hypothalamus and to observe effects within the hindbrain of those hypothalamic stimuli.

It is also known that there are strong neuroanatomical connections projecting from the nucleus of the solitary tract to the PVN. Considerable data suggest that this is an important pathway for the reporting of information about peripheral metabolism to central regulatory sites, including the PVN.

These observations make it clear that the neural systems regulating food intake and energy metabolism are distributed and interconnected. They are distributed in the sense that important regulation can take place at more than one neural site and at more than one neural projection. There is evidence indicating a linkage between processing sites; the PVN and the NTS at minimum, for example, are linked in a reciprocal and interconnected way so that somehow they participate jointly in management of energy balance. It appears likely that this distributed, interconnected characteristic identified for the NPY example is a general characteristic of the neural regulation of feeding.

This example interrelating NPY in the PVN with opioids in the NTS is also an example of how different neurotransmitter systems might be interconnected, and of how disparate signals may be processed to yield the ultimate output variables, such as feeding. What is not yet clear is whether or not the NTS functions as a site for the apparent property of reward, which is associated with opioid-induced feeding. To whatever extent such a property exists, it represents a means of studying interaction between, for example, energy deprivation and desire with respect to food intake.

The hypothalamus and the hindbrain are probably not the only important brain sites involved in the neural networks that regulate feeding and energy metabolism. There is already considerable evidence for other brain sites, such as the amygdala and, particularly, the central nucleus of the amygdala, as well as the cortex, in these neuroregulatory systems. An understanding of how these various processing sites interconnect and interplay in order to produce the observed behavioral and metabolic effects represents the great challenge of the immediate future.

Literature Cited

1. Akabayashi A, Watanabe Y, Wahlestedt C, McEwen BS, Paez X, Leibowitz SF. 1994. Hypothalamic neuropeptide Y, its gene expression and receptor activity: relation to circulating corticosterone in adrenalectomized rats. *Brain Res.* 665:201–12
2. Akil H, Watson SJ, Young E, Lewis ME, Khachaturian H, Walker JM. 1984. Endogenous opioids: biology and function. *Annu. Rev. Neurosci.* 7:223–55
3. Arjune D, Bowen WD, Bodnar RJ. 1991. Ingestive behavior following central [D-Ala2, Leu5, Cys6]-enkephalin (DALCE), a short-acting agonist and long-acting antagonist at the delta opioid receptor. *Pharmacol. Biochem. Behav.* 39:429–36
4. Arjune D, Standifer KM, Pasternak GW, Bodnar RJ. 1990. Reduction by central beta-funaltrexamine of food intake in rats under freely-feeding, deprivation and glucoprivic conditions. *Brain Res.* 535:101–9
5. Bartness TJ, Warren WS, Adkinson MG. 1996. Sympathetic nervous system innervation of white adipose tissue: from the hypothalamus to the fat pad. *Obes. Res.* 4:S63
6. Bergmann F, Lieblich I, Cohen E, Ganchrow JR. 1985. Influence of intake of sweet solutions on the analgesic effect of a low dose of morphine in randomly bred rats. *Behav. Neural Biol.* 44:347–53
7. Berman Y, Devi L, Carr KD. 1994. Effects of chronic food restriction on prodynorphin-derived peptides in rat brain regions. *Brain Res.* 664:49–53
8. Berman Y, Devi L, Carr KD. 1995. Effects of streptozotocin-induced diabetes on prodynorphin-derived peptides in rat brain regions. *Brain Res.* 685:129–34
9. Bertino M, Beauchamp GK, Engelman K. 1991. Naltrexone, an opioid blocker, alters taste perception and nutrient intake in humans. *Am. J. Physiol.* 261:R59–R63
10. Billington CJ, Briggs JE, Grace M, Levine AS. 1991. Effects of intracerebroventricular injection of neuropeptide Y on energy metabolism. *Am. J. Physiol.* 260:R321–27
11. Billington CJ, Briggs JE, Harker S, Grace M, Levine AS. 1994. Neuropeptide Y in hypothalamic paraventricular nucleus: a center coordinating energy metabolism. *Am. J. Physiol.* 266:R1765–70
12. Billington CJ, Levine AS. 1996. Appetite regulation: shedding new light on obesity. *Curr. Biol.* 6:920–23
13. Blass E, Fitzgerald E, Kehoe P. 1987. Interactions between sucrose, pain and isolation distress. *Pharmacol. Biochem. Behav.* 26:483–89
14. Bodnar RK. 1996. Opioid receptor subtype antagonist and ingestion. In *Opioid Receptor Subtype Antagonist and Ingestion*, ed. SJ Cooper, PG Clifton, pp. 127–46. London: Academic
15. Bossone CA, Hannon JP. 1991. Metabolic actions of morphine in conscious chronically instrumented pigs. *Am. J. Physiol.* 29:R1051–57
16. Brady LS, Smith MA, Gold PW, Herkenham M. 1990. Altered expression of hypothalamic neuropeptide mRNAs in food-restricted and food-deprived rats. *Neuroendocrinology* 52:441–47
17. Brennan G, Bachus SE, Jhanwar-Uniyal M. 1994. Involvement of gene expression for hypothalamic dynorphin in dietary preference. *J. Neurosci.* 20:818
18. Britton DR, Indyk E. 1989. Effects of ganglionic blocking agents on behavioral responses to centrally administered CRF. *Brain Res.* 478:205–10
19. Britton DR, Koob GF, Rivier J, Vale W. 1982. Intraventricular corticotropin-releasing factor enhances behavioral effects of novelty. *Life Sci.* 31:363–67
20. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. 1995. Recombinant Mouse OB Protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* 269:546–47
21. Carr KD. 1984. The physiology of opiate hedonic effects and the role of opioids in motivated behavior. *Adv. Alcohol Subst. Abuse* 3:5–18
22. Carr KD. 1996. Opioid receptor subtypes and stimulation-induced feeding. In *Opioid Receptor Subtypes and Stimulation-Induced Feeding*, ed. SJ Cooper, PG Clifton, pp. 167–92. London: Academic
23. Carr KD, Simon EJ. 1983. Effects of naloxone and its quaternary analogue on stimulation-induced feeding. *Neuropharmacology* 22:127–30
24. Carr KD, Simon EJ. 1983. The role of opioids in feeding and reward elicited by lateral hypothalamic electrical stimulation. *Life Sci.* 1:563–66
25. Carr KD, Simon EJ. 1984. Potentiation of reward by hunger is opioid mediated. *Brain Res.* 297:369–73
26. Chua S Jr, Brown AW, Kim J, Hennessey KL, Leibel RL, Hirsch J. 1991. Food deprivation and hypothalamic neuropeptide

gene expression: effects of strain background and the diabetes mutation. *Brain Res. Mol. Brain Res.* 11:291–99
27. Clark JT, Kalra PS, Crowley WR, Kalra SP. 1984. Neuropeptide Y and human pancreatic polypeptide stimulate feeding behavior in rats. *Endocrinology* 115:427–29
28. Cooper SJ, Jackson A, Kirkham TC, Turkish S. 1988. Endorphins, opiates and food intake. In *Endorphins, Opiates and Food Intake*, ed. RJ Rodgers, SJ Cooper, pp. 143–86. London: Wiley & Sons
29. Corder R, Pralong F, Turnill D, Saudan P, Muller AF, Gaillard RC. 1988. Dexamethasone treatment increases neuropeptide Y levels in rat hypothalamic neurons. *Life Sci.* 43:1879–86
30. Davies L, Marks JL. 1994. Role of hypothalamic neuropeptide Y gene expression in body weight regulation. *Am. J. Physiol.* 266:R1687–91
31. Dean RG, White BD. 1990. Neuropeptide Y expression in rat brain: effects of adrenalectomy. *Neurosci. Lett.* 114:339–44
32. Drewnowski A, Krahn DD, Demitrack MA, Nairn K, Gosnell BA. 1992. Taste responses and preferences for sweet high-fat foods: evidence for opioid involvement. *Physiol. Behav.* 51:371–79
33. Dube MG, Sahu A, Kalra PS, Kalra SP. 1992. Neuropeptide Y release is elevated from the microdissected paraventricular nucleus of food-deprived rats: an in vitro study. *Endocrinology* 131:684–88
34. Dum J, Gramsch C, Herz A. 1983. Activation of hypothalamic beta-endorphin pools by reward induced by highly palatable food. *Pharmacol. Biochem. Behav.* 18:443–47
35. Egawa M, Yoshimatsu H, Bray GA. 1991. Neuropeptide Y suppresses sympathetic activity to interscapular brown adipose tissue in rats. *Am. J. Physiol.* 260:R328–34
36. Egawa M, Yoshimatsu H, Bray GA. 1993. Effect of ß-endorphin on sympathetic nerve activity to interscapular brown adipose tissue. *Am. J. Physiol.* 264:R109–15
37. Fantino M, Hosotte J, Apfelbaum M. 1986. An opioid antagonist, naltrexone, reduces preference for sucrose in humans. *Am. J. Physiol.* 251:R91–R96
38. Farrell PA, Gates WK, Maksud MG, Morgan WP. 1982. Increases in plasma beta-endorphin/beta-lipotropin immunoreactivity after treadmill running in humans. *J. Appl. Physiol.* 52:1245–49
39. Glass MJ, Grace M, Cleary JP, Billington CJ, Levine AS. 1996. Potency of naloxone's anorectic effect in rats is dependent on diet preference. *Am. J. Physiol.* 271:R217–21
40. Grandison L, Guidotti A. 1977. Stimulation of food intake by muscimol and beta endorphin. *Neuropharmacology* 16:533–36
41. Greenberg BR. 1986. Predictors of binge eating in bulimic and nonbulimic women. *Int. J. Eating Disord.* 5:269–84
42. Gunion MW, Peters RH. 1981. Pituitary beta-endorphin, naloxone, and feeding in several experimental obesities. *Am. J. Physiol.* 241:R173–84
43. Gunion MW, Rosentahl MJ, Morley JE, Miller S, Zib B, et al. 1991. μ-Receptor mediates elevated glucose and corticosterone after third ventricle injection of opioid peptides. *Am. J. Physiol.* 261:R60–R81
44. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, et al. 1995. Weight-reducing effect of the plasma protein encoded by the obese gene. *Science* 269: 543–46
45. Harri M, Kuusela P. 1986. Is swimming exercise or cold exposure for rats? *Acta Physiol. Scand.* 126:189–97
46. Heilig M, McLeod S, Koob GK, Britton KT. 1992. Anxiolytic-like effect of neuropeptide Y (NPY), but not other peptides in an operant conflict test. *Regul. Pept.* 41:61–69
47. Heilig M, Widerlov E. 1995. Neurobiology and clinical aspects of neuropeptide Y. *Crit. Rev. Neurobiol.* 9:115–36
48. Heinrichs SC, Cole BJ, Pich EM, Menzaghi F, Koob GF, Hauger RL. 1992. Endogenous corticotropin-releasing factor modulates feeding induced by neuropeptide Y or a tail-pinch stressor. *Peptides* 13:879–84
49. Heinrichs SC, Koob GF. 1992. Corticotropin-releasing factor modulates dietary preference in nutritionally and physically stressed rats. *Psychopharmacology* 109:177–84
50. Hulsey MG, Pless CM, Martin RJ. 1995. ICV administration of anti-corticotropin-releasing factor antisense oligonucleotide: effects on feeding behavior and body weight. *Regul. Pept.* 59:241–46
51. Jewett DC, Cleary J, Levine AS, Schaal DW, Thompson T. 1992. Effects of neuropeptide Y on food-reinforced behavior in satiated rats. *Pharmacol. Biochem. Behav.* 42:207–12
52. Jewett DC, Cleary J, Levine AS, Schaal DW, Thompson T. 1995. Effects of neuropeptide Y, insulin, 2-deoxyglucose, and

food deprivation on food-motivated behavior. *Psychopharmacology* 120:267–71
53. Jewett DC, Schaal DW, Cleary J, Thompson T, Levine AS. 1991. The discriminative stimulus effects of neuropeptide Y. *Brain Res.* 561:165–68
54. Kalra SP, Dube MG, Sahu A, Phelps CP, Kalra PS. 1991. Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. *Proc. Natl. Acad. Sci. USA* 88:10931–35
55. Kaye WH, Gwirtsman HE, George DT, Weiss SR, Jimerson DC. 1986. Relationship of mood alterations to bingeing behaviour in bulimia. *Br. J. Psychiatry* 149:479–85
56. Kehoe P, Blass EM. 1986. Opioid-mediation of separation distress in 10-day-old rats: reversal of stress with maternal stimuli. *Dev. Psychobiol.* 19:385–98
57. Kim E, Welch CC, Grace MK, Billington CJ, Levine AS. 1996. Chronic food restriction and acute food deprivation decrease mRNA levels of opioid peptides in arcuate nucleus. *Am. J. Physiol.* 270:R1019–24
58. Kirchgessner AL, Sclafani A. 1988. PVN-hindbrain pathway involved in the hypothalamic hyperphagia-obesity syndrome. *Physiol. Behav.* 42:517–28
59. Kirkham TC, Blundell JE. 1984. Dual action of naloxone on feeding revealed by behavioural analysis: separate effects on initiation and termination of eating. *Appetite* 5:45–52
60. Kirkham TC, Blundell JE. 1986. Effect of naloxone and naltrexone on the development of satiation measured in the runway: comparisons with d-amphetamine and d-fenfluramine. *Pharmacol. Biochem. Behav.* 25:123–28
61. Kirkham TC, Blundell JE. 1987. Effects of naloxone and naltrexone on meal patterns of freely-feeding rats. *Pharmacol. Biochem. Behav.* 26:515–20
62. Kirkham TC, Cooper SJ. 1988. Naloxone attenuation of sham feeding is modified by manipulation of sucrose concentration. *Physiol. Behav.* 44:491–94
63. Kotz CM, Grace MK, Briggs JE, Levine AS, Billington CJ. 1995. Effect of opioid antagonists naloxone and naltrexone on neuropeptide Y-induced feeding and brown fat thermogenesis in the rat: neural site of action. *J. Clin. Invest.* 96:163–70
64. Krahn DD, Gosnell BA, Grace M, Levine AS. 1986. CRF antagonist partially reverses CRF- and stress-induced effects on feeding. *Brain Res. Bull.* 17:285–89
65. Krahn DD, Gosnell BA, Levine AS, Morley JE. 1988. Behavioral effects of corticotropin-releasing factor: localization and characterization of central effects. *Brain Res.* 443:63–69
66. Lefer L. 1971. Psychic stress and the oral cavity. *Postgrad. Med.* 49:171–75
67. Leibowitz SF. 1991. Brain neuropeptide Y: an integrator of endocrine, metabolic and behavioral processes. *Brain Res. Bull.* 27:333–37
68. Leibowitz SF. 1994. Specificity of hypothalamic peptides in the control of behavioral and physiological processes. *Ann. NY Acad. Sci.* 739:12–35
69. Levin BE. 1995. Reduced norepinephrine turnover in organs and brains of obesity-prone rats. *Am. J. Physiol.* 268:R389–94
70. Levine AS, Billington CJ. 1991. Stress and feeding behavior. *Clin. Appl. Nutr.* 1:33–41
71. Levine AS, Billington CJ. 1996. Peptides in regulation of energy metabolism and body weight. In *Peptides in Regulation of Energy Metabolism and Body Weight*, ed. C Bouchard, GA Bray, pp. 180–91. London: Wiley & Sons
72. Levine AS, Grace M, Billington CJ. 1991. Beta-funaltrexamine (beta-FNA) decreases deprivation and opioid-induced feeding. *Brain Res.* 562:281–84
73. Levine AS, Grace M, Billington CJ, Portoghese PS. 1990. Nor-binaltorphimine decreases deprivation and opioid-induced feeding. *Brain Res.* 534:60–64
74. Levine AS, Kuskowski MA, Grace M, Billington CJ. 1991. Food deprivation-induced vs. drug-induced feeding: a behavioral evaluation. *Am. J. Physiol.* 260: R546–52
75. Levine AS, Morley JE. 1981. Peptidergic control of insulin-induced feeding. *Peptides* 2:261–64
76. Levine AS, Morley JE. 1984. Neuropeptide Y: a potent inducer of consummatory behavior in rats. *Peptides* 5:1025–29
77. Levine AS, Morley JE, Gosnell BA, Billington CJ, Bartness TJ. 1985. Opioids and consummatory behavior. *Brain Res. Bull.* 14:663–72
78. Levine AS, Morley JE, Raij L. 1986. Opiates and the anorexia of uremia. *Physiol. Behav.* 37:835–38
79. Levine AS, Murray SS, Kneip J, Grace M, Morley JE. 1982. Flavor enhances the antidipsogenic effect of naloxone. *Physiol. Behav.* 28:23–25
80. Levine AS, Rogers B, Kneip J, Grace M, Morley JE. 1983. Effect of centrally administered corticotropin releasing factor

(CRF) on multiple feeding paradigms. *Neuropharmacology* 22:337–39
81. Levine AS, Weldon DT, Grace M, Cleary JP, Billington CJ. 1995. Naloxone blocks that portion of feeding driven by sweet taste in food-restricted rats. *Am. J. Physiol.* 268:R248–52
82. Lewis DE, Shellard L, Koeslag DG, Boer DE, McCarthy HD, et al. 1993. Intense exercise and food restriction cause similar hypothalamic neuropeptide Y increases in rats. *Am. Physiol.* 264:E279–84
83. Lowy MT, Maickel RP, Yim GK. 1980. Naloxone reduction of stress-related feeding. *Life Sci.* 26:2113–18
84. Lynch WC. 1986. Opiate blockade inhibits saccharin intake and blocks normal preference acquisition. *Pharmacol. Biochem. Behav.* 24:833–36
85. Lynch WC, Grace M, Billington CJ, Levine AS. 1993. Effects of neuropeptide Y on ingestion of flavored solutions in nondeprived rats. *Physiol. Behav.* 54:877–80
86. Lynch WC, Hart P, Babcock AM. 1994. Neuropeptide Y attenuates satiety: evidence from a detailed analysis of patterns ingestion. *Brain Res.* 636:28–34
87. Malabu UH, Kilpatrick A, Ware M, Vernon RG, Williams G. 1994. Increased neuropeptide Y concentrations in specific hypothalamic regions of lactating rats: possible relationship to hyperphagia and adaptive changes in energy balance. *Peptides* 15:83–87
88. Mansour A, Fox CA, Akil H, Watson SJ. 1995. Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. *Trends Neurosci.* 18:22–29
89. Martin WR, Wikler A, Eades CG, Pescor FT. 1963. Tolerance to and physical dependence on morphine in rats. *Psychopharmacologia* 4:247–60
90. Menzaghi F, Heinrichs SC, Pich EM, Tilders FJ, Koob GF. 1993. Functional impairment of hypothalamic corticotropin-releasing factor neurons with immunotargeted toxins enhances food intake induced by neuropeptide Y. *Brain Res.* 618: 76–82
91. Miller DS, Mumford P, Stock MJ. 1967. Gluttony: thermogenesis in overeating man. *Am. J. Clin. Nutr.* 20:1223–29
92. Miller TW, Feibelman ND. 1989. Traumatic stress disorder: diagnostic and clinical issues in psychiatry. In *Traumatic Stress Disorder: Diagnostic and Clinical Issues in Psychiatry,* ed. TW Miller, pp. 553–71. Madison: Int. Univ. Press
93. Morien A, Wellman PJ, Fojt J. 1995. Diurnal rhythms of paraventricular hypothalamic norepinephrine and food intake in rats. *Pharmacol. Biochem. Behav.* 52:169–74
94. Morley JE. 1983. Neuroendocrine effects of endogenous opioid peptides in human subjects: a review. *Psychoneuroendocrinology* 8:361–79
95. Morley JE. 1990. Appetite regulation by gut peptides. *Annu. Rev. Nutr.* 10:383–95
96. Morley JE, Levine AS. 1980. Stress-induced eating is mediated through endogenous opiates. *Science* 209:1259–61
97. Morley JE, Levine AS. 1982. Corticotropin releasing factor, grooming and ingestive behavior. *Life Sci.* 31:1459–64
98. Morley JE, Levine AS, Gosnell BA, Kneip J, Grace M. 1987. Effect of neuropeptide Y on ingestive behaviors in the rat. *Am. J. Physiol.* 252:R599–609
99. O'Hare E, Cleary J, Billington CJ, Levine AS. 1996. Naloxone administration following operant training of sucrose/water discrimination in the rat. *Psychopharmacology. In press*
100. Ostrowski NL, Rowland N, Foley TL, Nelson JL, Reid LD. 1981. Morphine antagonists and consummatory behaviors. *Pharmacol. Biochem. Behav.* 14:549–59
101. Parker LA, Maier S, Rennie M, Crebolder J. 1992. Morphine- and naltrexone-induced modification of palatability: analysis by the taste reactivity test. *Behav. Neurosci.* 106:999–1010
102. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, et al. 1995. Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 269:540–42
103. Rainer K, Reinscheid RK, Nothacker HP, Bourson A, Ardati A, et al. 1995. Orphanin FQ: a neuropeptide that activates an opioidlike G protein-coupled receptor. *Science* 270:792–94
104. Reid LD. 1985. Endogenous opioid peptides and regulation of drinking and feeding. *Am. J. Clin. Nutr.* 42:1099–132
105. Rockwood GA, Reid LD. 1982. Naloxone modifies sugar-water intake in rats drinking with open gastric fistulas. *Physiol. Behav.* 29:1175–78
106. Rothwell NJ, Stock MJ, Warwick BP. 1985. Energy balance and brown fat activity in rats fed cafeteria diets or high-fat, semisynthetic diets at several levels of intake. *Metabolism* 34:474–80
107. Rudski JM, Thomas D, Billington CJ, Levine AS. 1995. Buprenorphine increases intake of freely available and operant-contingent food in satiated rats. *Pharmacol. Biochem. Behav.* 50:271–76

108. Sahu A, Kalra PS, Kalra SP. 1988. Food deprivation and ingestion induce reciprocal changes in neuropeptide Y concentrations in the paraventricular nucleus. *Peptides* 9:83–86
109. Sayetta RB. 1986. Pica: an overview. *Am. Fam. Physician.* 33:181–85
110. Schwartz MW, Figlewicz DP, Woods SC, Porte D Jr, Baskin DG. 1993. Insulin, neuropeptide Y, and food intake. *Ann. NY Acad. Sci.* 692:60–71
111. Seegmiller JE. 1980. Diseases of purine and pyrimidine metabolism. In *Diseases of Purine and Pyrimidine Metabolism*, ed. PK Bondy, LE Rosenberg, pp. 877–937. Philadelphia: Saunders
112. Seeley RJ, Benoit SC, Davidson TL. 1995. Discriminative cues produced by NPY do not generalize to the interoceptive cues produced by food deprivation. *Physiol. Behav.* 58:1237–41
113. Seeley RJ, Payne CJ, Woods SC. 1995. Neuropeptide Y fails to increase intraoral intake in rats. *Am. J. Physiol.* 268:R423–27
114. Shaw NS, Rutherdale M, Kenny J. 1985. Eating more and enjoying it less: U. S. prison diets for women. *Women. Health* 10:39–57
115. Shibasaki T, Yamauchi N, Kato Y, Masuda A, Imaki T, et al. 1988. Involvement of corticotropin-releasing factor in restraint stress-induced anorexia and reversion of the anorexia by somatostatin in the rat. *Life Sci.* 43:1103–10
116. Shide DJ, Blass EM. 1991. Opioid mediation of odor preferences induced by sugar and fat in 6-day-old rats. *Physiol. Behav.* 50:961–66
117. Sims EAH, Goldman RF, Gluck CM, Horton ES, Kelleher PC, Rowe DW. 1968. Experimental obesity in man. *Trans. Am. Assoc. Phys.* 81:153–60
118. Singhi P, Singhi S. 1983. Nutritional status and psycho-social stress in children with pica. *Indian Pediatr.* 20:345–49
119. Sipols AJ, Brief DJ, Ginter KL, Saghafi S, Woods SC. 1992. Neuropeptide Y paradoxically increases food intake yet causes conditioned flavor aversions. *Physiol. Behav.* 51:1257–60
120. Smith GP, Gibbs J. 1994. Satiating effect of cholecystokinin. *Ann. NY Acad. Sci.* 713:236–41
121. Smith MS. 1993. Lactation alters neuropeptide-Y and proopiomelanocortin gene expression in the arcuate nucleus of the rat. *Endocrinology* 133:1258–65
122. Spiess J, Rivier J, Rivier C, Vale W. 1981. Primary structure of corticotropin-releasing factor from ovine hypothalamus. *Proc. Natl. Acad. Sci. USA* 78:6517–21
123. Spina M, Merlo-Pich E, Chan RKW, Basso AM, Rivier J, Vale W, et al. 1996. Appetitie-suppressing effects of urocortin, a CRF-related neuropeptide. *Science* 273:1561–64
124. Stanley BG, Daniel DR, Chin AS, Leibowitz SF. 1985. Paraventricular nucleus injections of peptide YY and neuropeptide Y preferentially enhance carbohydrate ingestion. *Peptides* 6:1205–11
125. Stanley BG, Leibowitz SF. 1984. Neuropeptide Y: stimulation of feeding and drinking by injection into the paraventricular nucleus. *Life Sci.* 35:2635–42
126. Stephens TW, Basinski M, Bristow PK, Bue-Vallensky JM, Burgett SG, et al. 1995. The role of neuropeptide Y in the anti-obesity action of the obese gene product. *Nature* 377:530–32
127. Tempel DL, Leibowitz SF. 1993. Glucocorticoid receptors in PVN: interactions with NE, NPY, and Gal in relation to feeding. *Am. J. Physiol.* 265:E794–800
128. Tempel DL, Leibowitz SF. 1994. Adrenal steroid receptors: interactions with brain neuropeptide systems in relation to nutrient intake and metabolism. *J. Neuroendocr.* 6:479–501
129. Vale W, Spiess J, Rivier C, Rivier J. 1981. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* 213:1394–97
130. Vaswani K, Tejwani GA, Mousa S. 1983. Stress induced differential intake of various diets and water by rat: the role of the opiate system. *Life Sci.* 32:1983–96
131. Welch CC, Grace MK, Billington CJ, Levine AS. 1994. Preference and diet type affect macronutrient selection after morphine, NPY, norepinephrine and deprivation. *Am. J. Physiol.* 266:R426–33
132. Deleted in proof
133. Welch CC, Kim EM, Grace MK, Billington CJ, Levine AS. 1996. Palatability-induced hyperphagia increases hypothalamic dynorphin peptide and mRNA levels. *Brain Res.* 721:126–31
134. Weldon DT, O'Hare E, Cleary J, Billington CJ, Levine AS. 1996. Effect of naloxone on intake of cornstarch, sucrose and polycose diets in resticted and nonrestricted rats. *Am. J. Physiol.* 39:R1183–88
135. White BD, Dean RG, Edwards GL, Martin RJ. 1994. Type II corticosteroid receptor stimulation increases NPY gene ex-

pression in basomedial hypothalamus of rats. *Am. J. Physiol.* 266:R1523–29
136. White JD, Olchovsky D, Kershaw M, Berelowitz M. 1990. Increased hypothalamic content of preproneuropeptide-Y messenger ribonucleic acid in streptozotocin-diabetic rats. *Endocrinology* 126: 765–72
137. Wolinsky TD, Carr KD, Hiller JM, Simon EJ. 1994. Effects of chronic food restriction on mu and kappa opioid binding in rat forebrain: a quantitative autoradiographic study. *Brain Res.* 656:274–80
138. Yirmiya R, Lieblich I, Liebeskind JC. 1988. Reduced saccharin preference in CXBK (opioid receptor-deficient) mice. *Brain Res.* 438:339–42
139. Zarjevski N, Cusin I, Vettor R, Rohner-Jeanrenaud F, Jeanrenaud B. 1994. Intracerebroventricular administration of neuropeptide Y to normal rats has divergent effects on glucose utilization by adipose tissue and skeletal muscle. *Diabetes* 43: 764–69