Molecular Neurobiology

ISSN 0893–7648/07/35(1): 1–19/$30.00
ISSN (Online) 1559–1182

Molecular Signaling Involved in Regulating Feeding and Other Motivated Behaviors

Todd R. Gruninger,** Brigitte LeBoeuf**, Yishi Liu,** and L. Rene Garcia*

Department of Biology, Texas A&M University, TAMU 3258, College Station, TX

Abstract

The metabolic and nutritional status of an organism influences multiple behaviors in addition to food intake. When an organism is hungry, it employs behaviors that help it locate and ingest food while suppressing behaviors that are not associated with this goal. Alternatively, when an organism is satiated, food-seeking behaviors are repressed so that the animal can direct itself to other goal-oriented tasks such as reproductive behaviors. Studies in both vertebrate and invertebrate model systems have revealed that food-deprived and -satiated behaviors are differentially executed and integrated via common molecular signaling mechanisms. This article discusses cellular and molecular mechanisms for how insulin, neuropeptide Y (NPY), and serotonin utilize common signaling pathways to integrate feeding and metabolic state with other motivated behaviors. Insulin, NPY, and serotonin are three of the most well-studied molecules implicated in regulating such behaviors. Overall, insulin signaling allows an organism to coordinate proper behavioral output with changes in metabolism, NPY activates behaviors required for locating and ingesting food, and serotonin modulates behaviors performed when an organism is satiated. These three molecules work to ensure that the proper behaviors are executed in response to the feeding state of an organism.

Index Entries: Insulin; serotonin; neuropeptide Y; invertebrate behavior; vertebrate behavior.

Introduction

Both vertebrates and invertebrates have evolved various complex food-related behaviors to ensure survival and reproduction. Cellular and molecular strategies that coordinate food selection, food intake, and nutritional levels with other social and motivated behaviors are beginning to emerge. A key issue in understanding motivated behaviors is determining how different regions of the nervous system communicate with other tissues and organs to ensure appropriate behavioral output given

Received June 9, 2006; Accepted September 6, 2006.
*Author to whom correspondence and reprint requests should be addressed. E-mail: rgarcia@mail.bio.tamu.edu
**These authors contributed equally to this work.

Table 1
Behaviors Affected by Insulin, NPY, and Serotonin

Signaling molecule	Positive regulation	Negative regulation
Insulin	Learning and memory in vertebrates Male mating in *C. elegans*	Feeding in vertebrates and invertebrates
Neuropeptide Y	Feeding in vertebrates and invertebrates Locomotion in vertebrates	Food preference in *Drosophila* Aggression in vertebrates Social feeding in *C. elegans*
Serotonin	Feeding in vertebrates and invertebrates Male mating in *C. elegans* Aggression in vertebrates Appetite in invertebrates Avoidance of repellents in *C. elegans*	Feeding in vertebrates Sexual behavior in vertebrates Aggression in vertebrates

multiple stimuli. The organizational complexity of the vertebrate nervous system has rendered this goal a daunting but nonetheless tractable task. However, invertebrate models with less complex nervous systems, such as *Aplysia californica, Drosophila melanogaster*, and *Caenorhabditis elegans* show similar motivated behavioral states and can be used to reveal fundamental molecular signaling mechanisms involved.

Signals regulating feeding need to be coordinated with other behavioral states of the organism. Various signaling mechanisms believed to only regulate one aspect of food intake actually regulate multiple facets of this behavior. For example, insulin not only regulates glucose levels in the body but also influences appetite in conjunction with various other signaling molecules, including neuropeptide Y (NPY), leptin, dopamine, and serotonin *(1–6)*. All of these signaling molecules have multiple effects on brain function in addition to influencing other tissues and systems in the body. Both vertebrates and invertebrates use these signaling molecules to mediate changes in feeding state, and not surprisingly, these molecules have been reported to influence other motivated behaviors. This article focuses on three of the most studied signaling molecules implicated in regulating food intake—insulin, neuropeptide Y, and serotonin—and discusses their effects on other motivated behaviors (Table 1). We also highlight the similarities between systems and the utility of using invertebrate models to guide behavioral research in higher organisms.

Insulin

Control of Metabolism

Insulin's most widely studied role is its regulation of metabolism, including controlling the body's ability to handle food by internalizing glucose. Additionally, insulin regulates many other functions related to metabolism, such as reproduction and lifespan *(1,7)*. In vertebrates, insulin is synthesized in the pancreas and is released into the bloodstream as a result of increased blood glucose levels *(8–11)*. Insulin is then transported to fat and muscle cells, where it signals these cells to uptake glucose, thus lowering blood sugar levels (Fig. 1A; refs. *12* and *13*). The biological functions of insulin are mediated through the insulin receptor, and the signaling pathways regulated by this receptor are conserved across species. In vertebrates, insulin receptors are found in various tissues, including muscle, fat, liver, and brain cells, consistent with insulin's ability to control metabolism in a broad range of tissues

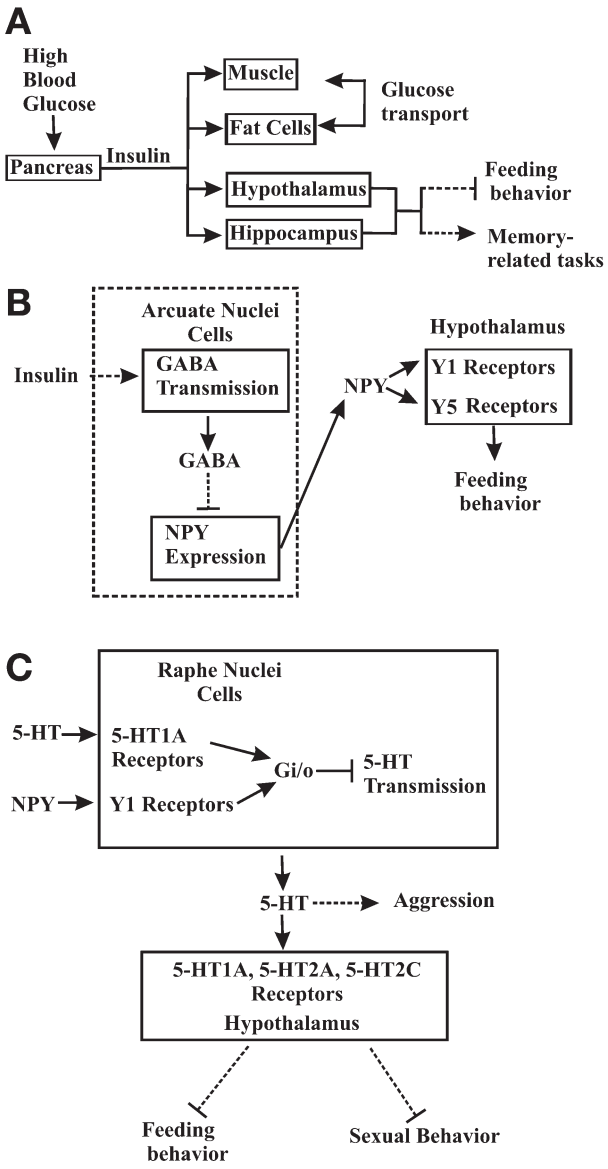

Fig. 1. Cellular and molecular mechanisms of insulin, NPY and serotonin regulation of behaviors in vertebrates. **(A)**, The squares depict cells in vertebrates that act in the insulin-signaling pathway. High blood glucose stimulates the pancreas to secrete insulin into general circulatory system (solid arrows). Insulin directly acts on insulin receptors on muscle and fat cells to uptake blood glucose (solid arrows). Insulin also acts on the insulin receptors of cells in the hypothalamus and hippocampus (solid arrows) to eventually attenuate feeding behaviors (dashed bar) and facilitate memory-related tasks (dashed arrow). **(B)**, Insulin inhibits NPY secretion from a population of cells in the Arcuate Nuclei. In the Arcuate Nuclei (dashed box), GABA-secreting cells (upper solid square) respond to insulin stimulation (dashed arrow). Secreted GABA leads to inhibition (dashed bar) of NPY synthesis in NPY-secreting cells (lower solid square). In the absence of insulin, NPY from the Arcuate Nuclei activates NPY receptors on other hypothalamic cells, which consequently promotes feeding behaviors. **(C)**, Activation of serotonin autoreceptors on serotonergic cells in the Raphe Nuclei can attenuate aggressive behavior. Serotonin from Raphe Nuclei cells can promote aggressive behavior (dashed arrow) and, through the hypothalamus, attenuate feeding and sexual behavior (dashed bars). Stimulation of serotonin autoreceptors or NPY receptors on the Raphe Nuclei serotonergic cells leads to downregulation of serotonin secretion (solid bar).

(14–17). The insulin receptor is a receptor tyrosine kinase composed of four subunits: two extracellular subunits that interact with the ligand and two intracellular subunits. When brought together, the intracellular subunits autophosphorylate, allowing docking of scaffolding proteins that activate kinases such as mitogen-activated protein kinase (MAPK) and phosphoinositide-3 kinase (PI3K) to regulate cell proliferation and glucose uptake, respectively *(18–20)*.

Insulin signaling also regulates metabolic processes in invertebrate model systems, including the nematode *C. elegans*. Mutations that affect the *C. elegans* homolog of the vertebrate insulin receptor DAF-2 result in adult animals with increased fat storage compared to wild-type controls *(7)*. DAF-2 signals via the conserved PI3K signal transduction cascade that results in phosphorylation of the transcription factor DAF-16/FOXO, inhibiting nuclear localization of the transcription factor

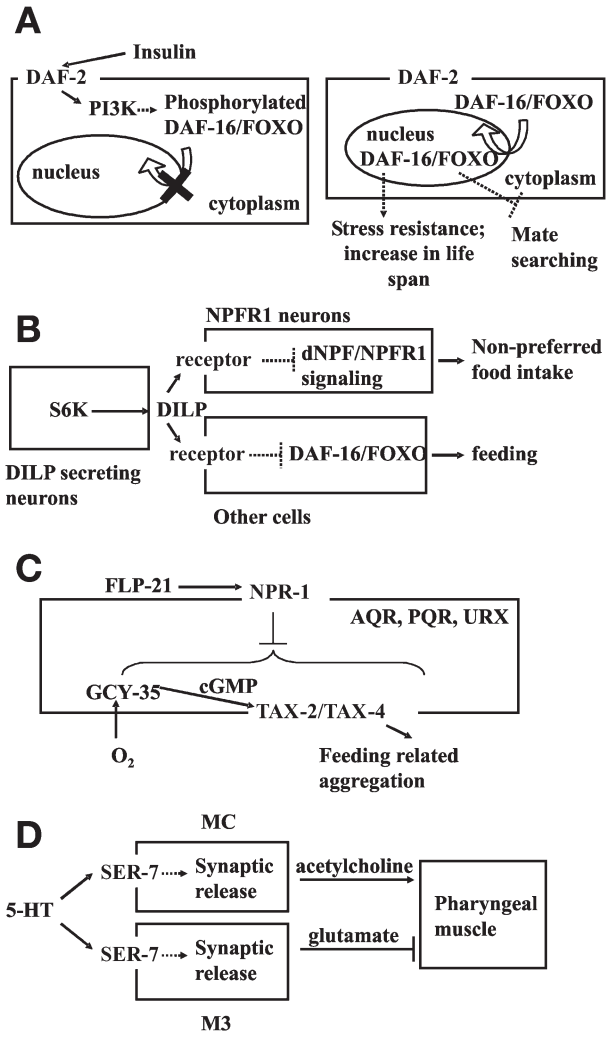

Fig. 2. Cellular and molecular mechanisms of insulin, NPY and serotonin regulation of behaviors in invertebrates. **(A),** The squares depict a general cell in *C. elegans* that responds to insulin-like signaling. The left cell shows that insulin signaling directly acts through the DAF-2 receptor and the PI3K kinase (solid arrows). Consequently, the DAF-16/FOXO transcription factor becomes phosphorylated (dashed arrow). Phosphorylated DAF-16/FOXO does not translocate into the nucleus. The left cell shows that when insulin signaling is reduced, nonphosphorylated DAF-16/FOXO translocates into nucleus. Therefore, low insulin signaling promotes stress resistance, life-span extension and reduces mate searching (dashed arrows). **(B)**, The squares depict cells in *Drosophila*. Ribosomal s6 kinase (S6K) positively regulates *Drosophila* insulin-like peptide (DILP) release from DILP secreting cells. DILP activates insulin receptors; this leads to inhibition of NPY/NPFR1 signaling (dashed bars), which then reduces nonpreferred food intake. In different cells, DILP can also negatively regulate DAF-16/FOXO (dashed bars) to inhibit feeding behavior. **(C)**, In *C. elegans* AQR, PQR, and URX neurons (generalized as the square) promote oxygen-induced aggregation behavior via the GCY-35/TAX-2/TAX-4 pathway (solid arrows). Neuropeptide FLP-21 stimulated NPR-1 receptors inhibit aggregation behavior through antagonizing this pathway in the same set of neurons (dashed bar). **(D),** In the *C. elegans* pharynx, MC and M3 motor neurons (depicted as squares in the left part of the figure) stimulate rhythmic pharyngeal muscle contractions (depicted as the square in the right part of the figure). Acetylcholine from MC activates pharyngeal muscle excitation; glutamate from M3 terminates pharyngeal muscle excitation. 5-HT increases the frequency of pharyngeal muscle action potentials by acting on SER-7 receptor on MC and M3 neurons. Activated SER-7 might increase MC and M3 neurons activity indirectly (dashed arrows).

(21–23). In the absence of DAF-2, nuclear-localized DAF-16 results in stress resistance and an increase in the lifespan of the organism (Fig. 2A; refs. *24* and *25*). Mutations in DAF-16 suppress increased fat storage of DAF-2 mutants, suggesting that fat metabolism in *C. elegans* is controlled via the conserved insulin receptor signaling cascade *(22,26,27)*.

Insulin-like signaling in *C. elegans* has also been shown to integrate cell metabolism with lifespan, stress response, and reproduction *(7)*. In addition to increased fat storage, loss of DAF-2 function results in adult animals that live twice as long as wild-type *(7,25)*. In larval animals, insulin-like signaling also regulates lifespan by controlling the nematode's development into a long-lived, nonfeeding state called the dauer larva. Normally, high levels of an insulin-like molecule indicate abundant food, and the dauer pathway is repressed. When

food is not scarce, low levels of this ligand initiate the dauer pathway, which allows the organism to survive in adverse environments. Decreased DAF-2 signaling, either because of starved conditions or mutation in the receptor, results in animals that develop into dauers *(7,28)*. The long-lived and constitutive dauer phenotypes of DAF-2 mutants depend on the activity of DAF-16, implicating the requirement of other conserved insulin signal transduction pathway components that also regulate fat metabolism.

Additionally, work in *C. elegans* has implicated that signals coming from the germline affect longevity, connecting the reproductive processes of an organism with its lifespan *(29)*. Therefore, insulin-like signaling in *C. elegans* not only acts to control the metabolic status of the organism but also integrates other biological processes, including metabolism, lifespan, and reproduction. Insulin's control of these processes is not just specific to invertebrates but is also apparent in higher organisms. For example, knocking out insulin receptors from fat cells in transgenic mice results in animals that have lower fat mass and live 18% longer than control mice *(30)*.

Control of Behaviors

Once insulin was found to pass the blood-brain barrier and insulin receptors were identified in the vertebrate central nervous system, insulin's role in modulating behavior became an active area of study *(15,31–33)*. Two areas of the brain found to have high concentrations of insulin receptors are the hypothalamus (an area involved in regulating animal activity, emotional behavior, sexual behavior, and metabolism) and the hippocampus (an area involved in learning and memory) (Fig. 1A; ref. *15*). Based on brain lesion studies in rats, these areas have been suggested to regulate behavioral responses to food. Chemical ablation of the hypothalamus results in greater food intake and obesity *(34)*. Conversely, hippocampal lesions cause rats to eat the same amount of food as control animals but consume much smaller amounts per meal and eat larger numbers of meals *(35)*. Insulin might control how these two brain regions affect food consumption because knocking out the insulin receptor in the central nervous system causes mice to increase feeding behavior *(1)*.

In the hypothalamus and hippocampus, studies have found a correlation between insulin levels and behaviors associated with learning and memory. Mice who are fed at regular intervals eventually express higher levels of insulin in the hypothalamus immediately prior to meal-time, and if food is not administered at the usual time, then hypothalamic insulin levels increase further *(36)*. This suggests that the animal may associate signals in the environment with food, and upregulate insulin levels at this time to prepare for feeding. In the hippocampus, insulin levels are also correlated with memory-like behaviors. This was demonstrated using the Morris water maze, which requires a swimming rat to learn the location of a platform submerged underwater. Rats that have been trained to locate the platform have increased levels of insulin receptors in the hippocampus, suggesting that insulin signaling is upregulated during learning *(37)*. In a similar study, diabetic rats required more time than control rats to learn where a camouflaged platform was located. However, when insulin was administered to these diabetic animals, the ability to locate the platform was restored *(38)*. Insulin appears to play a similar role in memory in humans, because increasing insulin levels increases memory in patients with Alzheimer's *(39,40)*.

The role of insulin in feeding behavior has also been investigated in invertebrate model systems. Studies of the *Drosophila* insulin-like receptor have found that insulin signaling can negatively regulate hunger-driven behaviors, including feeding rate and food preference. Similarly to *C. elegans*, *Drosophila*'s genome contains one identified insulin-like receptor that is involved in controlling growth, body size, lifespan, and sexual development *(41–44)*. To study how insulin influences feeding,

insulin signaling was disrupted in flies to determine if they still displayed normal feeding habits. Normally, well-fed *Drosophila* larvae avoid food that has been made noxious with the bitter compound quinine. Conversely, larvae that have been food-deprived eat the altered food. When insulin receptors are selectively removed from *Drosophila* neurons, well-fed larvae eat food regardless if it contains quinine or is unaltered, suggesting that food preference of well-fed larvae is regulated by insulin signaling in the nervous system. Consistent with this, overexpressing *Drosophila* insulin-like peptides (DILPs) in larvae results in starved animals that avoid noxious food *(45)*.

The amount of DILP secretion is controlled by the ribosomal s6 kinase (S6K); when S6K is upregulated in neurons that express DILPs, larvae show a decreased response to food, suggesting that S6K suppresses hunger response by positively regulating DILP release (Fig. 2B; ref. *46*). Therefore, S6K activity may decrease insulin signaling to upregulate feeding or food foraging behaviors when the animal is starved. Consistent with this, globally overexpressing the DAF-16/FOXO transcription factor, which is negatively regulated by insulin, results in larvae that display behaviors associated with starvation. Therefore, insulin signaling through the conserved DAF-16/FOXO signaling pathway mediates both behavioral and metabolic responses to food (Fig. 2B; ref. *47*).

In addition to insulin's regulation of feeding, some studies suggest insulin plays a role in human sexual behavior. Erectile dysfunction (ED) has long been known to be a side effect of men with diabetes, possibly because diabetes has many debilitating effects on the body *(48,49)*. However, one study posits that diabetes-related ED might also be correlated with reduced sexual libido *(50)*. However, because metabolism and behavior are integrated within a complex network, it is difficult to determine whether insulin's effect on human sexual psychology is direct.

Interestingly, a more direct link between insulin signaling and male sexual behavior has been found in *C. elegans* male mate-searching behavior *(51)*. This behavior is initiated when males are placed on a food source that does not contain potential mating partners (hermaphrodites). Well-fed males leave the food source in search of potential mates elsewhere. Conversely, males that have been previously food-deprived do not display mate-searching behavior and instead stay at the food source. Reduction of insulin signaling via a mutation in the *C. elegans* insulin-like receptor DAF-2 results in well-fed males that do not engage in mate-searching, mimicking starved males. The behavioral defect caused by reduced DAF-2 signaling can be suppressed by reducing DAF-16/FOXO function, thus demonstrating that the conserved insulin pathway can integrate the nutritional status of the organism with motivational behaviors such as male mating (Fig. 2A; ref. *51*).

Neuropeptide Y

Regulation of Feeding

Similarly to insulin, neuropeptide Y (NPY) plays a crucial role in regulating energy homeostasis, food intake, and social behavior in a variety of organisms; however, its cellular effects are mediated through a G protein-coupled receptor (NPY-R) rather than a receptor tyrosine kinase. In vertebrates, NPY exerts its effects through activating multiple NPY receptors; for example, five different NPY receptors, Y1, Y2, Y4, Y5 and Y6, have been cloned in humans, rats, and mice *(52–57)*. In vitro studies using vertebrate cells have shown that activated NPY receptors transmit their signals through the pertussis toxin-sensitive G proteins, $G_{i/o}$ *(58,59)*. Models for how NPY uses such signaling pathways to regulate feeding and other related behaviors have been revealed for both vertebrates and invertebrates.

In vertebrates, NPY signaling modulates behaviors associated with acquiring and consuming nutrients. NPY receptors Y1 and Y5 are expressed in the hypothalamus, the area of the

brain implicated in regulating motivated feeding and metabolism (Fig. 1B; refs. *53* and *60*). Intracerebroventricular injections of NPY into the hypothalamus stimulate feeding and, over time, can result in obese animals *(61–64)*. Exogenously added NPY is believed to stimulate Y1 and Y5 because mice with these receptors knocked out have reduced or eliminated behavioral response to NPY treatments. Conversely, mice lacking NPY or the Y1 and Y5 NPY receptors, do not display major feeding defects or abnormal body weight *(65–67)*. However, following a period of fasting, NPY knockout mice do show reduced responses to food *(68)*. These results suggest that NPY and the Y1 and Y5 NPY receptors are not essential when food is readily available to animals but might be used to modulate feeding behavior when food is scarce.

To ensure NPY-mediated behaviors are only displayed under proper conditions, NPY signaling pathways are linked with insulin-signaling pathways. Studies using diabetic rats showed that decreased insulin causes increased hypothalamic NPY expression and increased feeding behavior *(2)*. In a related study, when exogenous insulin was injected into fasted rats with low amounts of endogenous insulin, NPY levels normalized *(3)*. These results suggest that insulin negatively regulates feeding behavior by attenuating NPY expression and secretion. Insulin's regulation of NPY activity has been proposed to occur in the arcuate nucleus region of the hypothalamus, because NPY messenger RNA expression is attenuated by exogenous insulin in arcuate nucleus cell cultures. Additionally, this inhibitory effect requires synaptic transmission from GABAergic cells in the culture, indicating that insulin acts upstream of NPY-expressing cells (Fig. 2B; ref. *69*).

Behavioral studies in invertebrates such as *Drosophila* have provided further insight into how NPY signaling modifies feeding behavior. In *Drosophila* larvae, the homologs of NPY and NPYR1, neuropeptide F (dNPF), and neuropeptide F receptor 1 (NPFR1) have been implicated in regulating a behavioral switch in food acceptance or rejection. Developing larvae are attracted to food, and this is likely a result of the requirement to increase their body mass before pupation. Conversely, older larvae that have approached the time of pupation show decreases in feeding behavior. Correspondingly, dNPF RNA levels in the brain are high in developing larvae, whereas RNA levels decrease in older larvae, suggesting that dNPF signaling positively regulates feeding behavior, similarly to NPY signaling in vertebrates *(70)*. In addition to positively regulating feeding in developing larva, dNPF signaling also downregulates food preference in *Drosophila*. Feeding larvae that have not been food-deprived prefer to eat food in liquid rather than in solid form. However, overexpressing NPFR1 results in indiscriminate feeding, causing the larvae to show no preference to one type of food over the other *(45,46)*. These results suggest dNPF signaling may be employed to ensure the organism feeds when food sources are scarce. Therefore, NPFR1-expressing neurons could mediate responses to food as an organism develops and has different nutritional requirements *(70)*.

Intriguingly, studies that investigated how dNPF signaling regulates food preference in feeding larvae also found that this molecule controls avoidance to noxious food, a motivated behavior that is regulated by insulin. Normally, well-fed *Drosophila* larvae avoid food that has been mixed with quinine; however, fasted larvae are more willing to eat the noxious food. Conversely, knocking down NPFR1 in fasted larvae causes the larvae to avoid the noxious food. Quinine aversion in feeding larvae is also regulated by insulin signaling, suggesting that dNPF levels may be influenced by insulin, as NPY is in vertebrates. This is supported by the observation that a dominant-negative version of the insulin receptor expressed in NPFR1-expressing neurons causes well-fed larvae to eat noxious food *(45,46)*. Therefore, dNPF signaling appears to attenuate avoidance to noxious food when the metabolic status and insulin levels of the animal are stressed (Fig. 2B).

Regulation of Other Motivated Behaviors

NPY signaling has also been implicated in mediating other motivated behaviors, including anxiety and stress-related responses in vertebrates. The highest concentration of NPY neurons in mice is located in the basolateral nucleus of the amygdala (BLA), which is believed to be a key regulator of fear and anxiety behaviors *(71,72)*. Recent studies have found that injection of NPY into the BLA blocks anxiety responses to different stress-response assays *(73)*. Normally, an animal that has been restrained for 30 min and then placed with a partner rat spends a significantly reduced amount of time interacting (i.e., grooming) with the other rat compared to nonstressed controls. However, pretreatment of NPY in the BLA results in restrained animals that interact with their partners at normal levels.

Another stress response assay is the two-floor choice test; rats that are injected with the pharmacological stressor urocortin I (Ucn) on one of the two floors spend significantly less time on the floor that Ucn was administered, compared to control rats that have been injected with a non-Ucn-containing vehicle. Conversely, NPY-pretreated rats spend approximately equal amounts of time on both floors when injected with Ucn. These results support the idea that NPY can attenuate specific behaviors when the organism is stressed, such as the dNPF-inhibition of food avoidance observed in starved *Drosophila* larvae.

In addition to regulating feeding, studies of the NPY Y1 and Y5 receptors have revealed that Y1 and Y5 may also regulate locomotion and addictive behaviors, respectively. Originally, the NPY Y1 receptor was believed to only affect feeding behavior because knockout mice gain more weight than control animals. When these mice have been studied in more detail, it has been evident that they also display reduced locomotion compared to control animals. Therefore, reduced energy expenditure in NPY Y1 knockout mice could explain the observed obesity and implicate a role for NPY in locomotion regulation *(74)*. Recently, the NPY Y5 was implicated in alcohol reinforcement in rodent models of alcoholism. Specifically, selective antagonism of the Y5 receptor decreased ethanol consumption and reinforcement, suggesting that NPY signaling pathways can contribute to abnormal displays of food- and stress-related behaviors *(75)*.

Signaling through the NPY receptor also regulates feeding-related social behaviors in invertebrates. In *Drosophila*, 90% of older larvae migrate to the border of a glucose-agar plate, and the majority (82%) of these migrating animals then engage in a social-clumping behavior. Because older larvae have naturally lower levels of dNPF, this suggests signaling through the *Drosophila* NPY-like receptor downregulates social behaviors. Consistently with the idea of dNPF signaling functioning to inhibit clumping social behavior, dNPF-deficient animals display premature social bordering behaviors, and overexpression of dNPF suppresses the social bordering behavior of older larvae *(70)*.

Studies in *C. elegans* have found that NPY receptor signaling directly controls a social-clumping behavior similar to *Drosophila* larvae. Although there is no homolog of an NPY-like ligand, *C. elegans* contain a homolog of the NPY receptor NPR-1, and signaling through this receptor via non-NPY-like neuropeptides regulates social feeding behaviors. Different isolates of *C. elegans* display variation in feeding behaviors: some feed in aggregates (social foragers), and others feed alone (solitary foragers). This dimorphic behavior is controlled by the *npr-1* gene. The standard laboratory N2 strain of *C. elegans* contains an *npr-1* allele (that encodes an NPR-1 protein with a valine at position 215) that promotes solitary foraging behavior; conversely, a naturally occurring allele that encodes a change of a single amino acid in the receptor (NPR-1 215F) results in aggregation behavior *(76)*.

The amino acid change in different NPR-1 proteins has been proposed to affect how the receptor activates its G protein and subsequent downstream effector pathways *(76,77)*. In *C. elegans*, NPR-1 is activated by two FMR-

Famide-related neuropeptides encoded by the *flp-18* and *flp-21* genes *(77)*. Null mutation in *npr-1* and overexpression of FLP-21 enhance aggregation behavior, suggesting that this might be the default behavior that is suppressed by NPR-1/FLP-21 signaling (Fig. 2C). In *Xenopus* ooyctes, FLP-21 can activate NPR-1 of solitary worms better than the protein from aggregating worms, implicating that receptors on solitary feeding animals might have greater affinity to the neuropeptide *(77,78)*.

High oxygen concentration is an environmental signal that triggers aggregation behavior in *C. elegans*, and NPR-1 signaling has also been proposed to inhibit this behavior. AQR, PQR, and URX are three sensory neurons that promote high-oxygen-induced aggregation behavior via the soluble guanylate cyclase GCY-35; these neurons also express NPR-1 *(79,80)*. Oxygen binds to GCY-35, inducing the enzyme to catalyze the formation of cyclic guanosine monophosphate (cGMP). This second messenger then activates a cGMP-gated cation channel consisting of the subunits TAX-2 and TAX-4 *(81–83)*. When activated by high oxygen, AQR, PQR, and URX instruct the worm to move toward regions of low oxygen (ref. *79*; Fig. 2C). To regulate this oxygen avoidance behavior, ligand-stimulated NPR-1 might antagonize GCY-35 TAX-4/TAX-2 signaling and repress the activity of oxygen sensing neurons; consequently, aggregates of worms can then disperse if their food source is depleted (Fig. 2C; refs. *79* and *80*). High oxygen might act as an environmental stressor to worms, and the finding that the *C. elegans* NPY-like receptor downregulates responses to oxygen is consistent with NPY-inhibition of anxiety behaviors in rodents.

Serotonin

Modulation of Feeding Behavior by Serotonin

Serotonin (5-HT) is one of the most ancient neurotransmitters, and it has been implicated in a wide range of behaviors, including feeding, mood, aggression, sleep, pain, learning, and sexual activity *(84–88)*. The cellular effects of serotonin are mediated by a variety of specific cell-surface receptors (seven distinct classes). The majority of these receptors belong to the G protein-coupled receptor family that transduces signals via guanine nucleotide binding regulatory proteins. The 5-HT_1 receptor class signals through the G protein G_i; 5-HT_2 receptors signal through G_q; 5-HT_4, 5-HT_6, and 5-HT_7 receptors signal through G_s; and the 5-HT_5 receptor, although its cognate G protein has not been identified, is proposed to inhibit cyclic adenosine monophosphate (cAMP) production *(89–98)*. The 5-HT_3 receptor, which is a ligand-gated ion channel, is the only known serotonin receptor that is not a G protein-coupled receptor *(99)*. With this diverse set of receptor types, serotonin can act as both an activator and an inhibitor, allowing it to regulate and integrate many different behaviors.

In vertebrates, serotonin appears to play a primarily inhibitory role in feeding behavior by acting in the hypothalamus. Exogenous 5-HT or drugs that stimulate serotonin signaling result in decreased food intake in free-fed and food-deprived rats *(4–6)*. Alternatively, drugs that antagonize serotonin receptor signaling increase food intake *(88,100–102)*. Intraperitoneal injections of 5-HT agonists and antagonists in rats have identified 5-HT G protein-coupled receptors involved in both suppressing and activating feeding behavior. Agonists specific for the 5-HT_{1B} and 5-HT_{1C} receptors decrease food intake and induce behaviors associated with food satiation such as exploratory behavior, grooming, and sleep *(103)*. Conversely, administration of agonists specific to the 5-HT_{1A} receptor appears to mediate the opposite response. In the Raphe nuclei, the main source of serotonergic neurons in the brain, administration of 5-HT_{1A} receptor agonists increases food intake by activating somatodendritic autoreceptors, which inhibit cell firing and reduce serotonergic transmission (Fig. 1C; refs. *104–109)*. It is not currently known how 5-HT_{1A} signaling in the brain stem

coordinates with other serotonin receptor subtypes and other brain areas to modulate feeding. Overall, it has been proposed that serotonin signaling acts on the hypothalamus to control the satiation process by negatively regulating how long the animal feeds. This is supported by the findings that increased 5-$HT_{2A/2B}$ receptor signaling in the hypothalamus decreases the duration of individual meals but has no effect on the frequency of meals taken *(5,110,111)*.

Similarly to the vertebrate systems, serotonin appears to play a major role in modulating feeding behaviors in invertebrates. In *C. elegans*, serotonin regulates feeding behavior by controlling the sensitivity of the muscular organ responsible for feeding, the pharynx. Administration of exogenous serotonin results in increased pharyngeal muscle contraction and food intake, whereas chronic depletion of serotonin reduces pharyngeal contraction rate *(112–115)*. Similarly to insulin and NPY, serotonin appears to be modulatory, because serotonin-deficient animals can still contract their pharyngeal muscle and ingest food, albeit at a decreased efficiency *(115)*. Cell-ablation studies have found that the pharyngeal muscles have their own intrinsic firing capability, and the pharyngeal neurons M3 and MC serve to regulate timing and feeding efficacy *(116)*. The M3 motor neurons inhibit muscle contraction by releasing glutamate, and the MCs stimulate muscle contraction via the neurotransmitter acetylcholine (Fig. 2D; refs. *112* and *116–121)*. The increase in pharyngeal muscle contractions by serotonin requires synaptic transmission and the M3 and MC neurons, suggesting that the effect of 5-HT regulates feeding by affecting receptors on pharyngeal neurons *(120,122)*. Consistently, researchers have determined that 5-HT stimulation of the pharynx requires the *C. elegans* 5-HT_7-like G protein-coupled-receptor (SER-7), which is expressed in pharyngeal neurons MC and M3, but not in the pharyngeal muscles (Fig. 2D; ref. *123*). In humans, the 5-HT_7 receptor is expressed widely in the brain. It has been implicated in relaxation of smooth muscle, circadian rhythms, migraines, sensory processing, and regulation of limbic processes, although it is not entirely clear how it regulates these behaviors *(124,125)*.

In *Apylsia*, many classical studies have demonstrated the importance of serotonin in the formation of learning and memory, and recent findings have shown it also plays a role in feeding behavior. Feeding behavior in *Aplysia* consists of an anticipatory or appetitive phase during which the animal senses and seeks out food, followed by a physical feeding phase that includes repetitive biting and swallowing. Previous contact to food results in faster response time and subsequent biting, suggesting that the anticipatory state of the animal, or its appetite, can influence the feeding phase *(126–128)*. Both in vitro and in vivo studies suggest serotonergic neurons that are active during the appetitive phase play an important role in establishing the preparatory state for feeding behaviors *(129–131)*. It has been proposed that during the appetitive phase, serotonergic neurons are active and increase their synaptic output to motorneurons responsible for feeding *(131)*. Serotonergic input increases the sensitivity of these neurons to upstream food signals, causing them to stimulate muscles faster in the presence of food.

Serotonergic Integration of Feeding With Other Motivated Behaviors

In addition to regulating appetite and satiation, serotonin signaling pathways also regulate the proper timing and execution of other behaviors linked to feeding. Aggressive behavior is sometimes associated with feeding when organisms are competing for natural resources; in rodents, serotonin signal transduction pathways appear to regulate both feeding and aggression. Intraperitoneal injections of 5-HT receptor agonists have found that stimulation of 5-HT_1 reduces aggressive behavior, whereas stimulation of 5-HT_3 receptors leads to an increase in aggression *(132–134)*. It is not clear how serotonin regulates aggression in vertebrates at the neural and molecular levels,

because removal of serotonin signaling has broader effects than aggression. Historically, serotonin is believed to play a primarily inhibitory role in aggression because of the finding that depletion of serotonin in the Raphe nuclei leads to increased aggression *(135,136)*. However, recent studies on the 5-HT_{1A} receptor have challenged this view. In the Raphe nuclei, the benzodioxopiperazine drug S 15535 increases 5-HT_{1A} autoreceptor activity on serotonin secreting cells and simultaneously decreases 5-HT_{1A} activity on their postsynaptic partners *(137,138)*. Using S 15535, researchers determined that activating 5-HT_{1A} autoreceptors on serotonergic neurons in the Raphe nuclei decreases aggressive behavior. This suggests that 5-HT_{1A} autoreceptors in the Raphe nuclei act in presynaptic neurons to inhibit aggressive behavior by decreasing serotonergic neurotransmission (Fig. 1C; ref. *139*). Because serotonin signaling appears to both repress and facilitate aggression, it has been proposed that increased serotonin signaling activates normal responses of aggression, whereas abnormal responses of aggression occur when serotonin is significantly low *(140)*.

The finding that the 5-HT_{1A} receptor acts as an autoreceptor to regulate aggression is similar to its proposed role in feeding behavior, suggesting that 5-HT_{1A} signaling pathways may be involved in integrating these two behaviors. The finding that NPY appears to couple territorial aggression and feeding by acting on 5-HT_{1A}-expressing neurons in the Raphe nuclei is consistent with this idea. Loss of NPY signaling as a result of knocking out the Y1 receptor in mice results in increased territorial aggression, and this aggressive phenotype can be suppressed by administration of a 5-HT_{1A} receptor agonist *(141)*. It is possible that the feeding and aggression are regulated by the same intracellular pathways because both Y1 and 5-HT_{1A} receptors signal through $G_{i/o}$ (Fig. 1C).

Serotonin also regulates male mating behaviors in vertebrate model systems. Activating 5-HT_{2C} receptors facilitates erections and inhibits ejaculation, whereas pharmacological stimulation of 5-HT_{1A} facilitates ejaculation and represses sexual satiation *(142–144)*. Additionally, it has been proposed that high levels of serotonin after ejaculation may result in the observed quiescence and latency until the next mating attempt. This is supported by the finding that injection of selective serotonin reuptake inhibitors into the anterior lateral hypothalamic area increases the latency of males to copulate, and this effect is blocked by 5-HT_{2A} antagonists (Fig. 1C; refs. *145* and *146*). The role of serotonin signaling in reducing mating drive is similar to its role in satiation during feeding behavior; both of these motivated behaviors are modulated by 5-HT_{2A} receptor signaling in the hypothalamus. Therefore, it is possible that serotonin signaling may link sexual motivation with feeding status in vertebrates; it remains to be determined how these two behaviors are integrated at the cellular and molecular levels.

Similarly to vertebrates, behavioral studies in *C. elegans* have shown that serotonin signaling coordinates feeding with other food-dependent behaviors. These behaviors include, but are not limited to, locomotion, egg-laying, chemotaxis, and male mating. Serotonin regulates locomotion rate in response to the food status of the worm. Specifically, food-deprived animals display an "enhanced slowing response" by significantly decreasing their locomotion when they encounter food (bacteria). However, this behavioral response is defective in serotonin biosynthesis mutants and in animals lacking the serotonergic neurons in the pharynx, the NSMs *(147)*.

Serotonin levels and feeding status also regulate chemosensory avoidance in *C. elegans*. Normally, well-nourished worms avoid the chemical repellent octanol; 10 min of starvation results in a decreased response to the repellent. Endogenous serotonin is required for response of well-fed animals to octanol; mutants lacking the serotonin synthesis enzyme tryptophan hydroxylase mimic starved animals. In this case, it appears that serotonin acts directly on one pair of chemosensory neurons via the worm-specific G protein GPA-11 to mediate the change in sensitivity to the aversive chemical

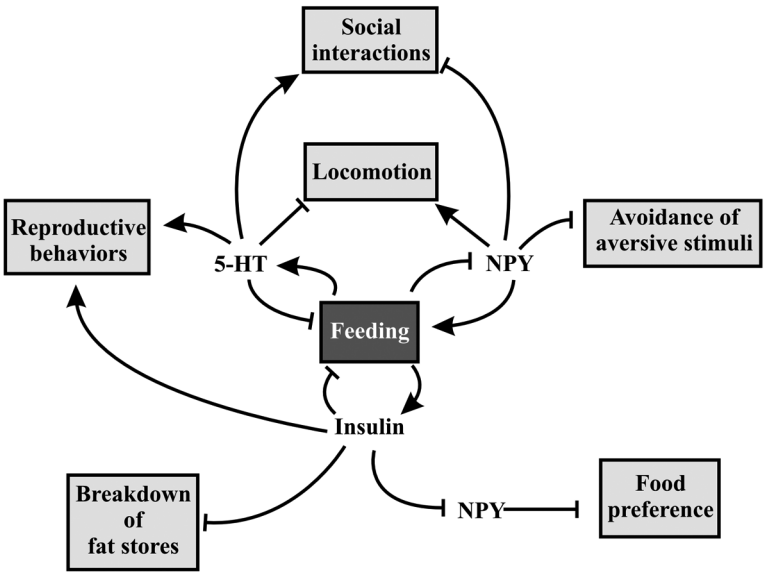

Fig. 3. Insulin, NPY, and serotonin (5-HT) integrate multiple behaviors associated with feeding state. A schematic diagram summarizing general behaviors (closed boxes) influenced by insulin, NPY and 5-HT is shown. Arrows indicate positive regulation of the behavior by the neurotransmitter, whereas bar-headed lines indicate negative regulation. Both insulin and serotonin signaling are activated by food intake and negatively regulate behaviors associated with low food intake and positively regulate reproductive and social behaviors. Conversely, NPY levels rise in response to decreased food intake; NPY negatively regulates social and environmental responses and positively regulates locomotion and feeding to ensure the animal acquires and ingests food.

octanol. This behavioral change has been explained by the idea that food deprivation in starving animals relaxes responses to aversive stimuli, which allows the animal to search for food in nonpreferred environments *(148)*. The effect of serotonin on chemosensation in starved and nonstarved animals parallels the effects of insulin and NPY on *Drosophila* larval feeding, consistent with the idea that various signaling molecules regulate motivated behaviors via common intracellular pathways.

Feeding status and serotonin levels also affect *C. elegans* male mating behaviors. For example, the insulin-regulated mate-searching behavior discussed earlier also requires serotonin signaling, because serotonin-deficient males show a significant decrease in mate-searching behavior, similarly to starved males *(51)*. Feeding state and mating behavior may be integrated via the NSM pharyngeal neurons, which express serotonin and other neuropeptides that could signal the presence of food to the rest of the animal *(113,115, 117,149,150)*. When a male is on a food source, the pharynx rapidly contracts to ingest bacteria, but when he locates a hermaphrodite and initiates copulation, the pharyngeal contractions are repressed, a response that is dependent on the NSM neurons. Additionally, males that are food-deprived or contain mutations that affect muscle contractile proteins in the pharynx have reduced sex muscle excitability and are less likely to engage in mating, a phenotype that can be suppressed by ablating the NSM neurons. These results suggest that the NSMs can downregulate the male genitalia in response to pharyngeal signals and can regulate pharyngeal muscle contractions in response to sexual stimulation *(151)*. It is possible that this pair of neurons use various signaling molecules such as serotonin to regulate feeding and mating behaviors positively when

food is abundant as well as other neuropeptides to negatively regulate these behaviors when food is scarce. Therefore, individual circuits may coordinate the proper timing of feeding and other motivated behaviors by utilizing different signaling molecules.

Conclusion

Both vertebrates and invertebrates appear to utilize insulin, NPY, and serotonin signal transduction pathways to regulate behaviors that are associated with or are dependent on the presence of food in the environment (Fig. 3). These systems are coordinated to ensure proper behavioral responses to external and internal conditions. Insulin signaling mainly coordinates behaviors with energy homeostasis and metabolism; NPY signaling is involved in regulating behaviors that are required to ensure survival under poor nutrient conditions; and serotonin signaling appears to modulate behaviors that should be displayed during nutrient-rich conditions. Dietary intake is likely correlated with the synthesis of insulin, NPY, and serotonin, which makes these molecules ideal for modulating other behaviors that should be performed according to food accessibility. Using a small set of such signaling molecules, an organism can coordinate the proper timing of different behaviors without having to utilize multiple intracellular pathways for each behavior. However, mistakes in modulating a diverse range of behaviors with a few key regulators can lead to complex behavioral dysfunctions, such as the eating disorders anorexia and bulimia nervosa *(152-154)*. In vertebrates, the organizational complexity of the nervous system makes it difficult to gain a detailed understanding of neural and molecular principles of signal transduction and how to treat such conditions. This can be seen most readily in humans, where the drive to eat is not only influenced by hunger or satiation but is also affected by psychological and cultural aspects. Because serotonin, insulin, NPY, and other molecules regulate the same areas of the brain in vertebrates, it is likely they are modulating the same intracellular pathways to regulate motivated feeding behaviors. Invertebrate models such as *Drosophila* and *C. elegans* allow a detailed analysis of these signal transduction pathways within a less complex nervous system. Such studies can reveal basic principals of nervous system design and offer new direction for vertebrate models.

Acknowledgments

T.G, B.L, Y.L and L.R.G were supported by the Public Health Service grant, GM070431-1 and by the Searle Scholars Program.

References

1. Bruning J. C., et al. (2000) Role of Brain Insulin Receptor in Control of Body Weight and Reproduction. *Science* **289,** 2122–2125.
2. McKibbin P. E., et al. (1992) Insulin deficiency is a specific stimulus to hypothalamic neuropeptide Y: a comparison of the effects of insulin replacement and food restriction in streptozocin-diabetic rats. *Peptides* **13,** 721–727.
3. Schwartz M. W., et al. (1992) Inhibition of hypothalamic neuropeptide Y gene expression by insulin. *Endocrinology* **130,** 3608–3616.
4. Wong D. T., Reid L. R., and Threlkeld P. G. (1988) Suppression of food intake in rats by fluoxetine: comparison of enantiomers and effects of serotonin antagonists. *Pharmacol Biochem Behav*. **31,** 475–479.
5. Shor-Posner G., et al. (1986) Hypothalamic serotonin in the control of meal patterns and macronutrient selection. *Brain Res Bull*. **17,** 663–671.
6. Gibson, E. L., Kennedy A. J., and Curzon G. (1993) d–Fenfluramine- and d-norfenfluramine-induced hypophagia: differential mechanisms and involvement of postsynaptic 5-HT receptors. *Eur J Pharmacol*. **242,** 83–90.
7. Kimura, K. D., et al. (1997) daf-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans. *Science* **277,** 942–946.
8. Banting F. G. and Best C. H. (1922) The internal secretion of the pancreas. *J. Lab. Clin. Med.* **7,** 251–266.

9. Anderson E. and Long J. A. (1947) Suppression of insulin secretion by the growth hormone of the anterior pituitary as determined with the isolated rat pancreas in a perfusion apparatus. *Endocrinology* **40,** 92.
10. Grodsky G. M., et al. (1963) Effects of Carbohydrates on Secretion of Insulin from Isolated Rat Pancreas. *Am J Physiol*. **205,** 638–644.
11. Banting F. G. and Best C. H. (1922) Pancreatic extracts. *J. Lab. Clin. Med.* **7,** 464–472.
12. Crofford O. B. and A. E. Renold. (1965) Glucose Uptake by Incubated Rat Epididymal Adipose Tissue. Rate-Limiting Steps and Site of Insulin Action. *J Biol Chem*. **240,** 14–21.
13. Crofford O. B. and Renold A. E. (1965) Glucose Uptake by Incubated Rat Epididymal Adipose Tissue. Characteristics of the Glucose Transport System and Action of Insulin. *J Biol Chem*. **240,** 3237–3244.
14. Freychet P., Roth J., and Neville D. M. Jr. (1971) Insulin receptors in the liver: specific binding of (125 I) insulin to the plasma membrane and its relation to insulin bioactivity. *Proc Natl Acad Sci U S A*. **68,** 1833–1837.
15. Havrankova J., Roth J., and Brownstein M. (1978) Insulin receptors are widely distributed in the central nervous system of the rat. *Nature* **272,** 827–829.
16. Renner, R. (1973) Evidence for an insulin receptor in muscle. *FEBS Lett*. **32,** 87–90.
17. Kono T. (1969) Destruction of insulin effector system of adipose tissue cells by proteolytic enzymes. *J Biol Chem*. **244,** 1772–1778.
18. Ruderman N. B., et al. (1990) Activation of phosphatidylinositol 3 kinase by insulin. *Proc Natl Acad Sci U S A*. **87,** 1411–1415.
19. Kasuga M., et al. (1983) Tyrosine-specific protein kinase activity is associated with the purified insulin receptor. *Proc Natl Acad Sci U S A*. **80,** 2137–2141.
20. Okada T., et al. (1994) Essential role of phosphatidylinositol 3-kinase in insulin–induced glucose transport and antilipolysis in rat adipocytes. Studies with a selective inhibitor wortmannin. *J Biol Chem*. **269,** 3568–3573.
21. Lin K., et al. (1997) daf-16: An HNF-3/forkhead Family Member That Can Function to Double the Life-Span of Caenorhabditis elegans. *Science*. **278,** 1319–1322.
22. Ogg S., et al. (1997) The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. *Nature* **389,** 994–999.
23. Lin K., et al. (2001) Regulation of the Caenorhabditis elegans longevity protein DAF-16 by insulin/IGF-1 and germline signaling. *Nat Genet*. **28,** 139–145.
24. Henderson S. T. and Johnson T. E. (2001) daf-16 integrates developmental and environmental inputs to mediate aging in the nematode Caenorhabditis elegans. *Current Biol*. **11,** 1975–1980.
25. Kenyon C., et al. (1993) A *C. elegans* mutant that lives twice as long as wild type. *Nature* **366,** 461–464.
26. Ashrafi K., et al. (2003) Genome-wide RNAi analysis of Caenorhabditis elegans fat regulatory genes. *Nature* **421,** 268–272.
27. Ogg S. and Ruvkun G. (1998) The C. elegans PTEN homolog, DAF–18, acts in the insulin receptor-like metabolic signaling pathway. *Mol Cell*. **2,** 887–893.
28. Riddle D. (1977) A genetic pathway for dauer larva formation in *C. elegans. Stadler Genetics Symposium*. **9,** 101–120.
29. Hsin H. and Kenyon C. (1999) Signals from the reproductive system regulate the lifespan of C. elegans. *Nature*. **399,** 362–366.
30. Bluher M., Kahn B. B., and Kahn C. R. (2003) Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* **299,** 572–574.
31. Havrankova J., et al. (1978) Identification of insulin in rat brain. *Proc Natl Acad Sci U S A*. **75,** 5737–5741.
32. Woods S. C. and Porte D. Jr. (1977) Relationship between plasma and cerebrospinal fluid insulin levels of dogs. *Am J Physiol Endocrinol Metab*. **233,** E331–E334.
33. Margolis R. U. and Altszuler N. (1967) Insulin in the Cerebrospinal Fluid. *Nature*. **215,** 1375,1376.
34. Shimizu N., et al. (1987) Hyperphagia and obesity in rats with bilateral ibotenic acid-induced lesions of the ventromedial hypothalamic nucleus. *Brain Res*. **416,** 153–156.
35. Clifton P. G., Vickers S. P., and Somerville E. M.. (1998) Little and Often: Ingestive Behavior Patterns Following Hippocampal Lesions in Rats. *Behav. Neurosci*. **112,** 502–511.
36. Orosco M., et al. (1995) Feeding-related immunoreactive insulin changes in the PVN-VMH revealed by microdialysis. *Brain Res*. **671,** 149–158.
37. Zhao W., et al. (1999) Brain Insulin Receptors and Spatial Memory. Correlated changes in gene expression, tyrosine phosphorylation, and signaling molecules in the hippocampus of water maze trained rats. *J. Biol. Chem.* **274,** 34,893–34,902.
38. Biessels G. -J., et al. (1998) Water maze learning and hippocampal synaptic plasticity in

streptozotocin-diabetic rats: effects of insulin treatment. *Brain Res*. **800,** 125-135.
39. Craft S., et al. (1993) Effects of hyperglycemia on memory and hormone levels in dementia of the Alzheimer type: a longitudinal study. *Behav Neurosci*. **107,** 926–940.
40. Craft S., et al. (1996) Memory improvement following induced hyperinsulinemia in alzheimer's disease. *Neurobiol Aging*. **17,** 123–130.
41. Chen C., Jack J., and Garofalo R. S. (1996) The Drosophila insulin receptor is required for normal growth. *Endocrinology* **137,** 846–856.
42. Tatar M., et al. (2001) A mutant Drosophila insulin receptor homolog that extends lifespan and impairs neuroendocrine function. *Science* **292,** 107–110.
43. Ruan Y., et al. (1995) The Drosophila Insulin Receptor Contains a Novel Carboxyl-terminal Extension Likely to Play an Important Role in Signal Transduction. *J. Biol. Chem.* **270,** 4236–4243.
44. Bohni R., et al. (1999) Autonomous control of cell and organ size by CHICO, a Drosophila homolog of vertebrate IRS1-4. *Cell* **97,** 865–875.
45. Wu Q., Zhao Z., and Shen P. (2005) Regulation of aversion to noxious food by Drosophila neuropeptide Y- and insulin-like systems. *Nat Neurosci*. **8,** 1350–1355.
46. Wu Q., et al. (2005) Regulation of hunger-driven behaviors by neural ribosomal S6 kinase in Drosophila. *Proc Natl Acad Sci U S A*. **102,** 13,289–13,294.
47. Kramer J. M., et al. (2003) Expression of Drosophila FOXO regulates growth and can phenocopy starvation. *BMC Dev Biol*. **3,** 5.
48. Lustman P. J. and Clouse R. E. (1990) Relationship of psychiatric illness to impotence in men with diabetes. *Diabetes Care*. **13,** 893–895.
49. McCulloch D. K., et al. (1980) The prevalence of diabetic impotence. *Diabetologia*. **18,** 279–283.
50. Nakanishi S., et al. (2004) Erectile dysfunction is strongly linked with decreased libido in diabetic men. *Aging Male*. **7,** 113–119.
51. Lipton J., et al. (2004) Mate searching in Caenorhabditis elegans: a genetic model for sex drive in a simple invertebrate. *J Neurosci*. **24,** 7427–7434.
52. Larhammar D., et al. (1992) Cloning and functional expression of a human neuropeptide Y/peptide YY receptor of the Y1 type. *J Biol Chem*. **267,** 10,935–10,938.
53. Gerald C., et al. (1996) A receptor subtype involved in neuropeptide–Y–induced food intake. *Nature* **382,** 168–171.
54. Gerald C., et al. (1995) Expression cloning and pharmacological characterization of a human hippocampal neuropeptide Y/peptide YY Y2 receptor subtype. *J Biol Chem*. **270,** 26,758–26,761.
55. Bard J. A., et al. (1995) Cloning and functional expression of a human Y4 subtype receptor for pancreatic polypeptide, neuropeptide Y, and peptide YY. *J Biol Chem*. **270,** 26,762–26,765.
56. Weinberg D. H., et al. (1996) Cloning and expression of a novel neuropeptide Y receptor. *J Biol Chem*. **271,** 16,435–16,438.
57. Michel M. C., et al. (1998) XVI. International Union of Pharmacology recommendations for the nomenclature of neuropeptide Y, peptide YY, and pancreatic polypeptide receptors. *Pharmacol Rev*. **50,** 143–150.
58. Motulsky H. J. and Michel M. C. (1988) Neuropeptide Y mobilizes Ca2+ and inhibits adenylate cyclase in human erythroleukemia cells. *Am J Physiol*. **255,** E880–E885.
59. Ewald D. A., Sternweis P. C., and Miller R. J. (1988) Guanine nucleotide-binding protein Go–induced coupling of neuropeptide Y receptors to Ca2+ channels in sensory neurons. *Proc Natl Acad Sci U S A*. **85,** 3633–3637.
60. Kopp J., et al. (2002) Expression of the neuropeptide Y Y1 receptor in the CNS of rat and of wild-type and Y1 receptor knock-out mice. Focus on immunohistochemical localization. *Neuroscience* **111,** 443–532.
61. Stanley B. G., et al. (1989) Repeated hypothalamic stimulation with neuropeptide Y increases daily carbohydrate and fat intake and body weight gain in female rats. *Physiol Behav*. **46,** 173–177.
62. Kalra P. S., et al. (1997) Increased receptor sensitivity to neuropeptide Y in the hypothalamus may underlie transient hyperphagia and body weight gain. *Regul Pept*. **72,** 121–130.
63. Zarjevski N., et al. (1993) Chronic intracerebroventricular neuropeptide–Y administration to normal rats mimics hormonal and metabolic changes of obesity. *Endocrinology* **133,** 1753–1758.
64. Vettor R., et al. (1994) Induction and reversibility of an obesity syndrome by intracerebroventricular neuropeptide Y administration to normal rats. *Diabetologia* **37,** 1202–1208.
65. Levine A. S. and Morley J. E. (1984) Neuropeptide Y: A potent inducer of consummatory behavior in rats. *Peptides* **5,** 1025–1029.
66. Ste Marie L., et al. (2005) Modulation of neuropeptide Y expression in adult mice does not affect feeding. *Proc Natl Acad Sci U S A*. **102,** 18,632–18,637.

67. Marsh D. J., et al. (1998) Role of the Y5 neuropeptide Y receptor in feeding and obesity. *Nat Med*. **4,** 718–7-21.
68. Bannon A. W., et al. (2000) Behavioral characterization of neuropeptide Y knockout mice. *Brain Res*. **868,** 79–87.
69. Sato I., et al. (2005) Insulin inhibits neuropeptide Y gene expression in the arcuate nucleus through GABAergic systems. *J Neurosci*. **25,** 8657–8664.
70. Wu Q., et al. (2003) Developmental control of foraging and social behavior by the Drosophila neuropeptide Y-like system. *Neuron* **39,** 147–161.
71. Parker R. M. and Herzog H. (1999) Regional distribution of Y-receptor subtype mRNAs in rat brain. *Eur J Neurosci*. **11,** 1431–1448.
72. Wolak M. L., et al. (2003) Comparative distribution of neuropeptide Y Y1 and Y5 receptors in the rat brain by using immunohistochemistry. *J Comp Neurol*. **464,** 285–311.
73. Sajdyk T. J., Fitz S. D, and Shekhar A. (2006) The role of neuropeptide Y in the amygdala on corticotropin–releasing factor receptor-mediated behavioral stress responses in the rat. *Stress* **9,** 21–28.
74. Pedrazzini T., et al. (1998) Cardiovascular response, feeding behavior and locomotor activity in mice lacking the NPY Y1 receptor. *Nat Med*. **4,** 722–726.
75. Schroeder J. P., Overstreet D. H., and Hodge C. W. (2005) The neuropeptide-Y Y5 receptor antagonist L-152,804 decreases alcohol self-administration in inbred alcohol-preferring (iP) rats. *Alcohol*. **36,** 179–186.
76. de Bono M. and Bargmann C. I. (1998) Natural variation in a neuropeptide Y receptor homolog modifies social behavior and food response in C. elegans. *Cell* **94,** 679–689.
77. Rogers C., et al. (2003) Inhibition of Caenorhabditis elegans social feeding by FMR-Famide-related peptide activation of NPR-1. *Nat Neurosci*. **6,** 1178–1185.
78. Kubiak T. M., et al. (2003) Differential activation of "social" and "solitary" variants of the Caenorhabditis elegans G protein-coupled receptor NPR-1 by its cognate ligand AF9. *J Biol Chem*. **278,** 33,724–33,729.
79. Gray J. M., et al. (2004) Oxygen sensation and social feeding mediated by a C. elegans guanylate cyclase homologue. *Nature* **430,** 317–322.
80. Coates J. C. and de Bono M. (2002) Antagonistic pathways in neurons exposed to body fluid regulate social feeding in Caenorhabditis elegans. *Nature* **419,** 925–929.
81. Komatsu H., et al. (1999) Functional reconstitution of a heteromeric cyclic nucleotide-gated channel of Caenorhabditis elegans in cultured cells. *Brain Res*. **821,** 160–168.
82. Komatsu H., et al. (1996) Mutations in a cyclic nucleotide–gated channel lead to abnormal thermosensation and chemosensation in *C. elegans. Neuron* **17,** 707–718.
83. Coburn C. M. and Bargmann C. I. (1996) A putative cyclic nucleotide-gated channel is required for sensory development and function in *C. elegans. Neuron* **17,** 695–706.
84. Artigas F., et al. (1996) Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT1A antagonists. *Trends Neurosci*. **19,** 378–383.
85. Graeff F. G., et al. (1996) Role of 5-HT in stress, anxiety, and depression. *Pharmacol Biochem Behav*. **54,** 129–141.
86. Griebel G. (1995) 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. *Pharmacol Ther*. **65,** 319–395.
87. Meneses A. (1998) Physiological, pathophysiological and therapeutic roles of 5-HT systems in learning and memory. *Rev Neurosci*. **9,** 275–289.
88. Leibowitz S. F. and Alexander J. T. (1998) Hypothalamic serotonin in control of eating behavior, meal size, and body weight. *Biol Psych* **44,** 851–864.
89. Bard J. A., et al. (1993) Cloning of a novel human serotonin receptor (5-HT7) positively linked to adenylate cyclase. *J Biol Chem*. **268,** 23,422–23,426.
90. Carson M. J., et al. (1996) The 5HT5A serotonin receptor is expressed predominantly by astrocytes in which it inhibits cAMP accumulation: a mechanism for neuronal suppression of reactive astrocytes. *Glia* **17,** 317–326.
91. Zhang J. Y., Ashby C. R. Jr., and Wang R. Y. (1994) Effect of pertussis toxin on the response of rat medial prefrontal cortex cells to the iontophoresis of serotonin receptor agonists. *J Neural Transm Gen Sect*. **95,** 165–172.
92. Seuwen K., Magnaldo I., and Pouyssegur J. (1988) Serotonin stimulates DNA synthesis in fibroblasts acting through 5-HT1B receptors coupled to a Gi-protein. *Nature* **335,** 254–256.
93. Torres G. E., Arfken C. L., and Andrade R. (1996) 5-Hydroxytryptamine4 receptors reduce afterhyperpolarization in hippocampus by inhibiting calcium-induced calcium release. *Mol Pharmacol*. **50,** 1316–1322.
94. Erlander M. G., et al. (1993) Two members of a distinct subfamily of 5-hydroxytryptamine

receptors differentially expressed in rat brain. *Proc Natl Acad Sci U S A*. **90,** 3452–3456.
95. Shen Y., et al. (1993) Molecular cloning and expression of a 5-hydroxytryptamine7 serotonin receptor subtype. *J Biol Chem*. **268,** 18,200–18,204.
96. Ruat M., et al. (1993) Molecular cloning, characterization, and localization of a high-affinity serotonin receptor (5-HT7) activating cAMP formation. *Proc Natl Acad Sci U S A*. **90,** 8547-8551.
97. Cory R. N., et al. (1987) The 5-hydroxytryptamine (5-HT2) receptor stimulates inositol phosphate formation in intact and broken WRK1 cells: determination of occupancy-response relationships for 5-HT agonists. *J Pharmacol Exp Ther*. **241,** 258–267.
98. Stam N. J., et al. (1992) Genomic organization, coding sequence and functional expression of human 5-HT2 and 5-HT1A receptor genes. *Eur J Pharmacol*. **227,** 153–162.
99. Derkach V., Surprenant A., and North R. A. (1989) 5-HT3 receptors are membrane ion channels. *Nature*. **339,** 706–709.
100. Coscina D. V., et al. (1994) Intraventricular but not intraparaventricular nucleus metergoline elicits feeding in satiated rats. *Am J Physiol*. **266,** R1562–R1567.
101. Currie P. J. and Coscina D. V. (1996) Metergoline potentiates natural feeding and antagonizes the anorectic action of medical hypothalamic 5-hydroxytryptamine. *Pharmacol Biochem Behav*. **53,** 1023–1028.
102. Fletcher P. J. (1988) Increased food intake in satiated rats induced by the 5-HT antagonists methysergide, metergoline and ritanserin. *Psychopharmacology (Berl)*. **96,** 237–242.
103. Kitchener S. J. and Dourish C. T. (1994) An examination of the behavioural specificity of hypophagia induced by 5-HT1B, 5-HT1C and 5-HT2 receptor agonists using the post-prandial satiety sequence in rats. *Psychopharmacology (Berl)*. **113,** 369–377.
104. Fletcher P. J. (1991) Dopamine receptor blockade in nucleus accumbens or caudate nucleus differentially affects feeding induced by 8-OH-DPAT injected into dorsal or median raphe. *Brain Res*. **552,** 181–189.
105. Fletcher P. J. and Davies M. (1990) Dorsal raphe microinjection of 5-HT and indirect 5–HT agonists induces feeding in rats. *Eur J Pharmacol*. **184,** 265–271.
106. Dourish C. T., Hutson P. H., and Curzon G. (1985) Characteristics of feeding induced by the serotonin agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT). *Brain Res Bull*. **15,** 377–384.
107. Gilbert F. and Dourish C. T. (1987) Effects of the novel anxiolytics gepirone, buspirone and ipsapirone on free feeding and on feeding induced by 8-OH-DPAT. *Psychopharmacology (Berl)*. **93,** 349–352.
108. Hjorth S. and Magnusson T. (1988) The 5-HT 1A receptor agonist, 8-OH-DPAT, preferentially activates cell body 5-HT autoreceptors in rat brain in vivo. *Naunyn Schmiedebergs Arch Pharmacol*. **338,** 463–471.
109. Hutson P. H., Dourish C. T., and Curzon G. (1986) Neurochemical and behavioural evidence for mediation of the hyperphagic action of 8-OH-DPAT by 5-HT cell body autoreceptors. *Eur J Pharmacol*. **129,** 347–352.
110. Mancilla-Diaz J. M., et al. (2002) Effect of 5-HT in mianserin-pretreated rats on the structure of feeding behavior. *Eur Neuropsychopharmacol*. **12,** 445–451.
111. Blundell J. E. (1984) Serotonin and appetite. *Neuropharmacology*. **23,** 1537–1551.
112. Avery L. and Horvitz H. R. (1990) Effects of starvation and neuroactive drugs on feeding in *Caenorhabditis elegans*. *J Exp Zool*. **253,** 263–270.
113. Horvitz H. R., et al. (1982) Serotonin and octopamine in the nematode *Caenorhabditis elegans*. *Science* **216,** 1012–1014.
114. Croll N. A. (1975) Indolealkylamines in the coordination of nematode behavioral activities. *Can J Zool*. **53,** 894–903.
115. Sze J. Y., et al. (2000) Food and metabolic signalling defects in a Caenorhabditis elegans serotonin–synthesis mutant. *Nature* **403,** 560–564.
116. Avery L. and Horvitz H. R. (1989) Pharyngeal pumping continues after laser killing of the pharyngeal nervous system of *C. elegans*. *Neuron* **3,** 473–485.
117. Albertson D. G. and Thomson J. N. (1976) The pharynx of *Caenorhabditis elegans*. *Philos Trans R Soc Lond B Biol Sci*. **275,** 299–325.
118. Avery L., Bargmann C. I., and Horvitz H. R. (1993) The Caenorhabditis elegans unc–31 gene affects multiple nervous system–controlled functions. *Genetics* **134,** 455–464.
119. Dent J. A., Davis M. W., and Avery L. (1997) avr-15 encodes a chloride channel subunit that mediates inhibitory glutamatergic neurotransmission and ivermectin sensitivity in Caenorhabditis elegans. *Embo J*. **16,** 5867–5879.
120. Niacaris T. and Avery L. (2003) Serotonin regulates repolarization of the *C. elegans* pharyngeal muscle. *J Exp Biol*. **206,** 223–231.

121. Raizen D. M., Lee R. Y., and Avery L. (1995) Interacting genes required for pharyngeal excitation by motor neuron MC in Caenorhabditis elegans. *Genetics* **141,** 1365–1382.
122. Rogers C. M., et al. (2001) Regulation of the pharynx of *Caenorhabditis elegans* by 5-HT, octopamine, and FMRFamide-like neuropeptides. *J Neurobiol*. **49,** 235–244.
123. Hobson R. J., et al. (2006) SER-7, a Caenorhabditis elegans 5-HT7-like receptor, is essential for the 5-HT stimulation of pharyngeal pumping and egg laying. *Genetics* **172,** 159–169.
124. Vanhoenacker P., Haegeman G., and Leysen J. E. (2000) 5-HT7 receptors: current knowledge and future prospects. *Trends Pharmacol Sci*. **21,** 70–77.
125. Thomas D. R. and Hagan J. J. (2004) 5-HT7 receptors. *Curr Drug Targets CNS Neurol Disord*. **3,** 81–90.
126. Kupfermann I. (1974) Dissociation of the appetitive and consummatory phases of feeding behavior in Aplysia: a lesion study. *Behav Biol*. **10,** 89–97.
127. Kupfermann I. (1974) Feeding behavior in Aplysia: a simple system for the study of motivation. *Behav Biol*. **10,** 1–26.
128. Susswein A. J., Weiss K. R., and Kupfermann I. (1984) Internal stimuli enhance feeding behavior in the mollusc Aplysia. *Behav Neural Biol*. **41,** 90–95.
129. Kupfermann I., et al. (1979) Functional role of serotonergic neuromodulation in Aplysia. *Fed Proc*. **38,** 2095–2102.
130. Kupfermann I. and Weiss K. R. (1982) Activity of an identified serotonergic neuron in free moving Aplysia correlates with behavioral arousal. *Brain Res*. **241,** 334–337.
131. Proekt A. and Weiss K. R. (2003) Convergent mechanisms mediate preparatory states and repetition priming in the feeding network of Aplysia. *J Neurosci*. **23,** 4029–4033.
132. Ferris C. F., et al. (1997) Vasopressin/serotonin interactions in the anterior hypothalamus control aggressive behavior in golden hamsters. *J Neurosci*. **17,** 4331–4340.
133. Ricci L. A., Knyshevski I., and Melloni R. H. Jr. (2005) Serotonin type 3 receptors stimulate offensive aggression in Syrian hamsters. *Behav Brain Res*. **156,** 19–29.
134. Sijbesma H., Schipper J., and De Kloet E. R. (1990) The anti-aggressive drug eltoprazine preferentially binds to 5-HT1A and 5-HT1B receptor subtypes in rat brain: sensitivity to guanine nucleotides. *Eur J Pharmacol*. **187,** 209–223.
135. Golebiewski H. and Romaniuk A. (1985) The participation of serotoninergic system in the regulation of emotional-defensive behavior evoked by intrahypothalamic carbachol injections in the cat. *Acta Neurobiol Exp (Wars)*. **45,** 25–36.
136. Vergnes M., et al. (1988) Selective increase of offensive behavior in the rat following intrahypothalamic 5,7-DHT-induced serotonin depletion. *Behav Brain Res*. **29,** 85–91.
137. Meller E., Goldstein M., and Bohmaker K. (1990) Receptor reserve for 5-hydroxytryptamine1A-mediated inhibition of serotonin synthesis: possible relationship to anxiolytic properties of 5-hydroxytryptamine1A agonists. *Mol Pharmacol*. **37,** 231–237.
138. Millan M. J., et al. (1994) Novel benzodioxopiperazines acting as antagonists at postsynaptic 5-HT1A receptors and as agonists at 5-HT1A autoreceptors: a comparative pharmacological characterization with proposed 5-HT1A antagonists. *J Pharmacol Exp Ther*. **268,** 337–352.
139. de Boer S. F., et al. (2000) Somatodendritic 5-HT(1A) autoreceptors mediate the anti-aggressive actions of 5-HT(1A) receptor agonists in rats: an ethopharmacological study with S-15535, alnespirone, and WAY-100635. *Neuropsychopharmacology* **23,** 20–33.
140. de Boer S. F. and Koolhaas J. M. (2005) 5-HT1A and 5-HT1B receptor agonists and aggression: a pharmacological challenge of the serotonin deficiency hypothesis. *Eur J Pharmacol*. **526,** 125–139.
141. Karl T., et al. (2004) Y1 receptors regulate aggressive behavior by modulating serotonin pathways. *Proc Natl Acad Sci U S A*. **101,** 12,742–12,747.
142. Klint T. and Larsson K. (1995) Clozapine acts as a 5-HT2 antagonist by attenuating DOI-induced inhibition of male rat sexual behaviour. *Psychopharmacology (Berl)*. **119,** 291–294.
143. Matuszewich L., et al. (1999) Partial antagonism of 8-OH-DPAT'S effects on male rat sexual behavior with a D2, but not a 5-HT1A, antagonist. *Brain Res*. **820,** 55–62.
144. Rodriguez-Manzo G. and Fernandez-Guasti A. (1994) Reversal of sexual exhaustion by serotonergic and noradrenergic agents. *Behav Brain Res*. **62,** 127–134.

145. Lorrain D. S., et al. (1997) Extracellular serotonin in the lateral hypothalamic area is increased during the postejaculatory interval and impairs copulation in male rats. *J Neurosci*. **17,** 9361–9366.
146. Hull E. M., Muschamp J. W., and Sato S. (2004) Dopamine and serotonin: influences on male sexual behavior. *Physiol Behav*. **83,** 291–307.
147. Sawin E. R., Ranganathan R., and Horvitz H. R. (2000) *C. elegans* locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. *Neuron* **26,** 619–631.
148. Chao M. Y., et al. (2004) Feeding status and serotonin rapidly and reversibly modulate a Caenorhabditis elegans chemosensory circuit. *Proc Natl Acad Sci U S A*. **101,** 15,512–15,517.
149. Nathoo A. N., et al. (2001) Identification of neuropeptide-like protein gene families in Caenorhabditiselegans and other species. *Proc Natl Acad Sci U S A*. **98,** 14,000–14,005.
150. Kim K. and Li C. (2004) Expression and regulation of an FMRFamide-related neuropeptide gene family in *Caenorhabditis elegans*. *J Comp Neurol*. **475,** 540–550.
151. Gruninger T. R., et al. (2006) Integration of male mating and feeding behaviors in Caenorhabditis elegans. *J Neurosci*. **26,** 169–179.
152. Brewerton T. D. (1995) Toward a unified theory of serotonin dysregulation in eating and related disorders. *Psychoneuroendocrinology* **20,** 561–590.
153. Kaye W. H., et al. (2005) Serotonin alterations in anorexia and bulimia nervosa: new insights from imaging studies. *Physiol Behav*. **85,** 73–81.
154. Steiger H., et al. (2001) Implications of impulsive and affective symptoms for serotonin function in bulimia nervosa. *Psychol Med*. **31,** 85–95.