The neurogenetics and evolution of food-related behaviour

Scott J. Douglas, Ken Dawson-Scully and Marla B. Sokolowski

Department of Biology, University of Toronto, 3359 Mississauga Road, L5L 1C6, Canada

All organisms must acquire nutrients from the ambient environment to survive. In animals, the need to eat has driven the evolution of a rich array of complex food-related behaviours that ensure appropriate nutrient intake in diverse niches. Here, we review some of the neural and genetic components that contribute to the regulation of food-related behaviour in invertebrates, with emphasis on mechanisms that are conserved throughout various taxa and activities. We focus on synthesizing neurobiological and genetic approaches into a neurogenetic framework that explains food-related behaviour as the product of interactions between neural substrates, genes and internal and external environments.

Introduction to food-related behaviours

All life must acquire organic and inorganic substrates as fuel and raw material for energetic, metabolic and physiological processes. Whereas many plants synthesize their constituent organic compounds from carbon dioxide, water and sunlight, animals enact a heterotrophic strategy to gain necessary nutrients. In turn, they have evolved an impressive array of behaviours to detect, capture and ingest food. Broadly, food-related behaviour can be defined as actions elicited in response to hunger or the perception of food, therefore including food-searching activities, intake and post-ingestive behaviours. Multiple internal and external cues influence whether an animal is searching for food or eating at any given time. A major internal parameter is the level of hunger, which is governed by a milieu of regulatory factors interacting within an elaborate homeostatic system [1,2]. External factors include abundance and distribution of food, competitors, risk of predation, season and time of day. In humans, the decision to eat is complicated further by psychological and cultural factors. Owing to the breadth of contributing factors, food-related behaviour represents a rich interface that has been characterized from several different perspectives, including the ecological [3,4], physiological [5], neurobiological [6], psychological [7], endocrinological [8] and genetic [1,2].

A comprehensive description of any behaviour requires insight into the pattern and functioning of the neural circuits that underlie expression of the behaviour. Circuit output, which is often modifiable through experience, depends on parameters such as neuronal membrane properties, neuronal connections and modes of synaptic transmission. Each of these characteristics in turn emerges out of interactions between the environment and genes encoding the myriad of humoral, intercellular and intracellular factors that direct nervous system activity. Examples of molecular players important for representation of behaviour in the nervous system include signalling molecules, receptors, ion channels, intracellular transduction components, and regulators of transcription and protein synthesis.

In the past, the fields of behavioural neurobiology and behavioural genetics have advanced mostly separately, although both have benefited greatly from studies in invertebrates. Neurobiologists have used the small and tractable neural circuits of insects, crustaceans and molluscs, and geneticists have exploited *Drosophila melanogaster* and *Caenorhabditis elegans* for their short generation times, high fecundities, high-resolution genetic maps, sequenced genomes and ease of genetic manipulation. From both neural and genetic perspectives, a common theme emerging across invertebrates is the conservation of central regulators of feeding. Nevertheless, owing to the different systems employed in each field, comprehensive models of feeding behaviour have been elusive. For example, although the neuronal dynamics necessary for various ingestion and post-ingestion behaviours have been modelled exquisitely in some invertebrates, little is known of the genetic mechanisms that regulate the development and function of the underlying circuitry. By contrast, genetic analysis of food-related activities in other species has uncovered genes and pathways important for food-search and food-intake behaviours, but a neural context for gene function is lacking owing to the unavailability of circuit information.

In this article, we focus on integration, both mechanistically, in emphasizing the evolutionary conservation of neural and genetic regulators of food-related behaviour across invertebrate species, and practically, in proposing the uniting of neurobiological and genetic paradigms to build neurogenetic (see Glossary) models of feeding behaviour. For the former, invertebrate neural central pattern generators (CPGs) and intracellular cGMP-dependent signalling pathways are striking examples of conserved regulators. For the latter, the analysis of gene function in systems traditionally not associated with genetic techniques should help in understanding the relationships between genes, neural substrates and behaviour. As an example of how a neurogenetic synthesis

Corresponding author: Sokolowski, M.B. (msokolow@utm.utoronto.ca).
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might appear, we describe the regulation of food-related aggregation in *C. elegans*, where combined analysis of gene and neural function is beginning to set forth an integrative account of this behaviour.

Making connections: examining the neural basis of food-related behaviour

Elucidation of neural processes underlying natural behaviours has traditionally been achieved under the banner of neuroethology. Studies have often concentrated on small invertebrate circuits, leading to a wealth of knowledge about the neural basis for rhythmic behaviours such as respiration, locomotion and feeding. Systems used to model neural controls of feeding include the stomatogastric ganglion (STG) of the lobsters *Panulirus* and *Homarus* [9] and the crab *Cancer* [10], the buccal ganglia of the gastropods *Aplysia* and *Lymnaea* [11], and the subesophageal or frontal ganglia in insects such as the locust *Locusta* [12] or tobacco horn worm *Manduca sexta* [13]. These systems have been used to define how properties such as neuronal connections, neurotransmitter identities, membrane characteristics and inter-circuit communication contribute to specific feeding behaviours and to behavioural plasticity. One general finding has been the importance of neuronal pacemakers (i.e. CPGs) to the execution of various rhythmic feeding behaviours. CPGs act in diverse neurobiological contexts such as flight, respiration, locomotion and chewing, and use a conserved set of principles that includes prevalence of synaptic inhibition and an ability to modify output firing patterns depending on the state of the organism [10]. The manner in which CPG organization is transduced into invertebrate feeding activities has been meticulously exposited for several behavioural models, including rhythmic movement of the toothed radula during feeding in the sea slug *Aplysia* [14] (Figure 1).

Other studies have focused on cellular mechanisms underlying plasticity in CPG-controlled food-related behaviours. Jones and colleagues [15] showed using the pond snail *Lymnaea* that associative learning correlates
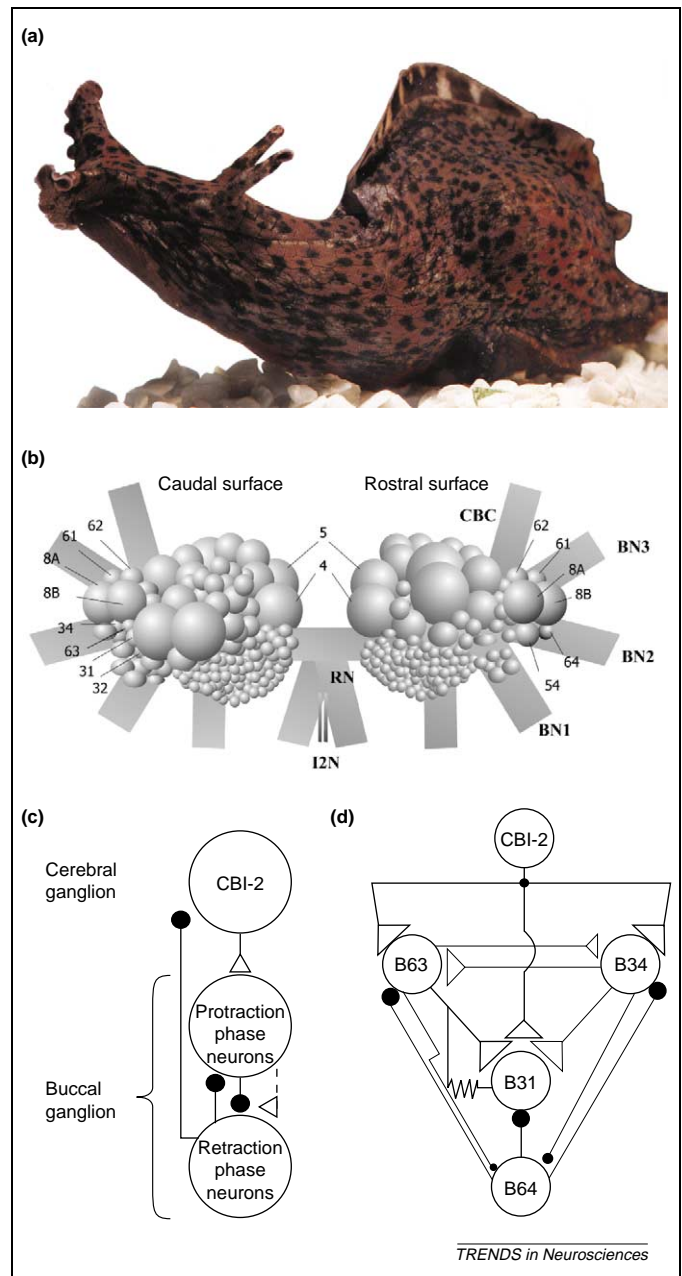

Figure 1. Morphological and neurological coupling of the central pattern generator (CPG) responsible for protraction and retraction movements of the toothed radula during feeding in *Aplysia*. Food touching the lips or stimulating the interior of the mouth of *Aplysia* initiates food intake, whereas maintained stimulation triggers repeated rhythmic protraction and retraction movements of the toothed radula. This rhythmic pattern is initiated by a command-like neuron, the cerebral–buccal interneuron 2 (CBI-2). **(a)** The marine mollusc *Aplysia*. **(b)** A morphological map of the rostral and caudal surfaces of the buccal ganglia, indicating the positions of neurons of the CPG involved in protraction and retraction of the radula during ingestion and egestion. Neurons of interest are numbered; nerves innervating the ganglia contain the letter N in their nomenclature. **(c)** Relationships among the command-like neuron CBI-2 of the cerebral ganglion and the protraction-phase and retraction-phase neurons in the buccal ganglion. Triangles represent excitatory coupling, dark circles represent inhibitory coupling and the broken line represents a polysynaptic connection. **(d)** A detailed circuit diagram of the neurons involved in the relationships between protraction and retraction phases, where CBI-2 stimulates the protraction CPG elements B63, B34 and B31 (and also B32, not shown in this figure). The retraction-phase is strongly governed by the CPG element B64, which inhibits all of the protraction-phase interneurons and is itself weakly inhibited by the protraction CPG elements. Triangles, dark circles and lines have the same meanings as in (c), with size corresponding to the strength of the specific connection; the 'resistor' (jagged line) represents electrical coupling. Panel (a) kindly provided by John H. Byrne; (b–d) modified, with permission, from [14].

with long-term depolarization of a key modulatory neuron in the circuits regulating ingestive movements. At the same time, phosphorylation of cAMP-response-element binding protein, an important modification for creation of long-term memory, is increased [16]. Another study showed in *Aplysia* that biting frequency can be enhanced through operant reward learning [17], a method that rewards an animal (in this case with an artificially invoked neural stimulus) for performing an initially neutral action. Once again, changes in the threshold required to produce action potentials in a key regulator of pacemaker activity correlated with behavioural plasticity. Therefore, both the internal dynamics of CPG networks related to food-related behaviours (Figure 1) and the integration of environmental inputs into such circuits have been modelled in invertebrates to provide an understanding of neural bases of feeding at the CPG level.

As mentioned, relatively little is known of the genetic mechanisms that underlie CPG function and plasticity in these invertebrate feeding systems. Nonetheless, the well-defined structure of the neural apparatus associated with feeding behaviours represents an ideal substrate for genetic analysis. Genetic characterization of these systems can identify key regulators of rhythmic behaviours, and the precise roles of these molecules in guiding the output of CPGs. An important question is how CPGs controlling food-related movements of an animal are customized to respond to nutrients in the internal or external environment. That is, do specialized circuit properties responsive to nutrient-related cues modulate a basic, conserved CPG mechanism to direct behaviour towards food-related activities? If so, which genes regulate basic CPG activity and how has the system been modified specifically to tie the neural substrates to food-related behaviour? Next, we present examples of how candidate-gene approaches and genomic techniques (see Glossary) might be used to dissect the genetics of feeding-related circuitries and address these issues. We then go on to provide an example of how genetic and neurobiological infrastructures can begin to be merged into a multi-tiered, integrative account of food-related behaviour.

Foraging ahead: discovering genes that regulate food-related behaviour

A central principle of classical genetic analysis is that the identification of genes regulating a biological trait requires heritable variation in the appearance or state of that trait. This phenotypic variation can occur naturally or result from experimentally induced mutations. For food-related behaviour, both natural gene variants and mutations that affect food-search patterns, types of foods preferred and amount eaten per meal have been identified [18–21]. As outlined below, analysing the molecular correlates of phenotypic variation is a powerful means of identifying novel genes and neural substrates important for expression of a behaviour.

Identification of the foraging gene in D. melanogaster
The term foraging describes both food-searching (appetitive) and food-intake (consummatory) processes, encompassing diverse activities that include intensive searching

in dogs, ranging in lions, 'theft' in scavengers and ambush strategies of spiders. Foraging behaviours can vary between genotypes within a species, over the life cycle of a single individual, and across environments. The first gene to be cloned that influences natural variation in food-related behaviour affects the foraging style of *D. melanogaster* larvae. Natural strains of these larvae exhibit one of two foraging patterns: 'rover' larvae travel further than 'sitter' larvae in a single patch of food and move more between food patches [18,22]. In adulthood, the strains retain their separate foraging strategies [23], and rovers also acquire an enhanced sensitivity to sucrose [24]. Notably, rover and sitter locomotion activities do not differ significantly in the absence of food. Genetic and molecular methods showed that the rover–sitter behavioural dimorphism is primarily due to variation at a single gene called *foraging* (*for*) that encodes a cGMP-dependent protein kinase (PKG). The finding that PKG levels are higher in rovers than in sitters, combined with a switch to rover-like movement patterns in transgenic sitters (see Glossary) overexpressing PKG, indicates that the difference in sitter appetitive behaviour is due to reduced PKG levels [25].

Although these data convincingly demonstrate a link between PKG levels and natural variation in larval foraging patterns, a neurogenetic understanding of the behaviour requires delineation of the circuitry components responsible for food-related movement. In addition, developmental, neuroanatomical and/or neurophysiological distinctions between rovers and sitters that account for behavioural differences should be investigated. As with CPGs, one challenge is to distinguish those aspects of the wiring that control general locomotion from those that mediate appetitive movements in response to food. The fact that rovers and sitters show similar movement patterns in the absence of food indicates that *for* must influence a food-specific pathway. However, the broad expression pattern of *for* and the requirement of PKG for diverse neurobiological processes [26] have made it difficult to identify relevant neurons.

Recent results have shown that the neural substrates important for male fly courtship behaviour are specified by a male-specific splice variant of the *fruitless* (*fru*) gene. *fru* is expressed in all peripheral sensory modalities important for courtship, and in at least one case (olfaction) is required to regulate the perception and response to courtship-relevant environmental cues. An intriguing aspect of *fru* function is that the circuitry it regulates, which is itself specific for courtship, appears to feed into general elements of the nervous system to direct appropriate mating behaviours [27,28]. Whether *for* acts in an analogous manner to affect circuitry that regulates food-related behaviour in *D. melanogaster* larvae remains to be seen. One possibility is that *for*, which expresses at least three transcript types, marks food-specific neural pathways with a single protein isoform. Similar to *fru*, *for* function could be associated with multiple circuits (e.g. those of taste, olfaction and/or gut chemosensors) that, when integrated, harness general locomotory machinery to effect the coordinated movement patterns that characterize the appetitive behaviour of a particular strain.

Given the many physiological factors influencing food-related behaviour, PKG might also act outside the nervous system, for example in the gut, fat bodies or ring gland, to influence larval movements and possibly other behaviours such as food intake. In any case, because PKG is not required to induce food-related behavioural changes (rovers, sitters and *for* mutants all alter their behaviours, albeit in different ways, when moved from non-nutritive media into the presence of food), other molecules no doubt act together with *for* within food-specific pathways to modify behaviour. Studies have begun to characterize how PKG affects cellular signalling and neuronal function in *D. melanogaster* [29,30], and future work on strains with mutations converting rovers into sitters [31] and sitters into rovers [32] should identify other molecules necessary for foraging.

Conservation of PKG function across multiple foraging behaviours

In developmental biology, an emerging theme is that principal regulators of morphology and differentiation have been re-used during evolution to generate novel patterns [33–36]. An intriguing question in behavioural research is whether related behaviours across phylogenetic taxa might also depend on similar molecules and cellular mechanisms. One way to address this issue is using a candidate-gene approach ([37] and references therein) to characterize the roles of genes such as *for* in the behaviours of other species. This has been done for the honeybee *Apis melifera*, a social insect in which young adults perform 'nursing' functions in the hive while older animals (2–3 weeks) forage for nectar and pollen outside the nest [38]. Ben-Shahar *et al.* [39] examined whether the transition from nurse to forager arises from differences in expression of the *for* honeybee cognate gene (*Amfor*). They found that foragers had enhanced levels of *Amfor* mRNA and PKG enzyme activity compared with nurses, even when bees were the same age. Furthermore, experimentally heightening PKG activity in young bees by treatment with cGMP induced precocious foraging, establishing a causative link between *Amfor* activity and appetitive behaviour.

That manipulation of PKG levels in both honeybees and fruitfly larvae is sufficient to spark dramatic changes in appetitive behaviours galvanizes the hypothesis that common regulators guide related behaviours across taxa. Remarkably, manipulation of PKG does not alter the general locomotion of honeybees or *D. melanogaster*, and treatment of nurse bees with the structurally similar cAMP produces no effect on foraging [39]. That *for* appears to regulate specifically the behavioural response to food in two divergent insect species implies that a cGMP pathway has been exploited during evolution to bridge the gap between the requirement for food and appropriate behavioural output.

These results show not only that PKG shares a function in the pathways leading to appetitive behaviours in bees and *D. melanogaster*, but also that variation in the levels of PKG in both species appears to account for natural differences in the expression of the behaviours. In *D. melanogaster*, the source of this variation is at the level of gene sequence: distinct alleles within populations are each associated with a different foraging strategy (rover or sitter). By contrast, in honeybees, single animals titre the level of PKG over their life cycles to regulate foraging activity. In this case, it is unknown whether the impetus provided by increased PKG levels specifically promotes foraging activities such as collecting nectar or whether it influences the nurse–forager transition more generally. It will be interesting to determine whether short-term flux of PKG activity is also used by *D. melanogaster* larvae to modify feeding behaviour. For example, both rovers and sitters reduce path lengths on food following food deprivation [40], raising the possibility that modulation of PKG levels in individual animals permits balancing of behavioural state with environmental or physiological conditions.

The natural variation observed for PKG levels between genotypes or during development does not necessarily occur for all gene products affecting food-related behaviour. Indeed, the great majority of genes do not vary or respond to change, but are necessary as part of the built-in infrastructure enabling the behaviour to proceed. The functions of such genes must be examined by assessing phenotypes of mutants that have altered gene sequences. For instance, in *C. elegans*, the *for* counterpart *egl-4* has not yet been associated with natural behavioural variation, but mutational analysis has revealed that here too PKG is required to regulate locomotion in foraging animals [19]. Again, *egl-4* does not appear to affect locomotion in the absence of food, indicating that the role of PKG as a specific regulator of appetitive behaviour is conserved in a non-insect species. Future studies should decipher whether PKG also affects foraging behaviour in mammals.

Like many gene products that affect behaviour, PKG has pleiotropic functions throughout the nervous system [26,41]. In *D. melanogaster*, null *for* mutations are lethal, and hypomorphic alleles impact foraging, habituation and sucrose responsiveness [24]. In *C. elegans*, by contrast, *egl-4* mutations alter foraging and olfactory adaptation, in addition to body size and lifespan [19,42,43]. It is unknown how PKG influences these various processes at the cellular and molecular levels, or how the molecule has become associated with seemingly distinct pathways across evolution. Equally unknown is to what extent different components of PKG pleiotropy, such as foraging and learning, are linked into common modules. Linkage could manifest as common use of the same neural substrates within an organism and maintenance of associations across evolution. One way to address these issues is through the use of microarrays, which should enable comparison of downstream consequences of PKG action in different tissues and species.

Identifying new genes required for food-related behaviours

In future, a candidate-gene approach could be used to investigate the degree of conservation of other gene functions involved in regulation of food-related behaviour. For example, genetic dissection of CPG function in animals such as *C. elegans*, *D. melanogaster* and zebrafish

has uncovered several important regulators of rhythmic activity [44], opening the door to candidate-gene studies of CPG-controlled feeding behaviours in other animals. In invertebrates such as crustaceans and molluscs that, as already discussed, have well-characterized CPG phenotypes, a candidate-gene approach could provide valuable insights into gene function that then help to elucidate the mechanistic basis of CPG function in other organisms, including *D. melanogaster*, *C. elegans* and mammals. In addition, it will be interesting to determine whether genes such as *for*, which up to now have been investigated in the context of appetitive foraging behaviour, also have a role in regulation of other food-related processes such as consummation, post-ingestion behaviours or energy homeostasis.

Besides genes previously identified in other species, another source of candidate genes is the microarray, which profiles changes in gene expression associated with different genetic backgrounds or environmental conditions. Complementary-DNA (cDNA) microarrays have been employed extensively to characterize RNA expression patterns in organisms with sequenced genomes, such as humans, *D. melanogaster* and yeast. However, by using expressed sequence tags (see Glossary) to construct cDNA libraries, genomics approaches have been extended to include organisms not traditionally associated with genetic analysis, including earthworms [45], carp [46], mussels [47] and gobies [48].

By detecting variation in gene activity between different conditions, microarrays can highlight specific genes as potential regulators of a process. For example, Zinke *et al.* [49] used expression profiles from food-deprived and carbohydrate-fed flies to identify a gene called *sugarbabe* (*sug*) that is activated in response to sugar ingestion. Other uses are diagnostic: Whitfield *et al.* [50] determined that honeybee nurses and foragers possess distinct transcript 'signatures' that highly correlate with behaviour. By comparing expression profiles of individual bees with those from known nurses and foragers, the identity of bees could be predicted with 95% accuracy.

One caveat of RNA-based microarrays is the assumption that protein levels within tissues are directly related to transcript abundance, which is not always the case. Thus, in addition to transcript profiling, proteomics methods [51] will help in uncovering the identities, structures and interactions of cellular proteins important for mechanisms of feeding behaviour. Like genetics, these genomics approaches enable identification of key genes and molecules that otherwise might not have been associated with food-related activities.

Of course, to confirm that a gene regulates a process of interest, causation must be established using manipulations that either repress gene function through genetic or post-transcriptional interference (see Glossary) or express gene products constitutively or ectopically using microinjection or transgenic techniques. Pharmacology, antisense technology and RNA interference (RNAi) represent important techniques for inhibiting gene function in organisms not amenable to traditional genetic approaches. For example, in *Lymnaea*, the enzyme nitric

oxide synthase (NOS) is expressed in key modulatory neurons of the feeding network, suggesting that it might be important during feeding [52]. To test this, Korneev *et al.* [53] performed RNAi targeted against NOS and found a lowered feeding response, indicating that NOS is required for feeding.

Development and application of transgenic techniques in a wider range of species will facilitate further dissection of the molecular mechanisms underlying feeding behaviour in invertebrates [54,55]. Transgenic methods confer fine control of neural activity by enabling repression or activation of specific nerve cells [56]. The recent production of 'remote control' flies exhibiting defined behaviours depending on the activity and position of photoactive ion channels demonstrates just how exact such technologies have become [57].

The neurogenetics of food-related behaviour: aggregation in *C. elegans*

Synthesis of a neurogenetic understanding of food-related behaviour has begun in the genetically tractable system *C. elegans*. Here, instead of superimposing gene function onto a pre-characterized neural phenotype, the general approach is to elucidate roles of genes side-by-side with functions of neurons, thereby gradually building up a comprehensive library of the genetic and neural machinery required for behaviour. Once the regulation and functioning of individual neurons has been established, they can be linked together into circuits that provide mechanistic models of food-related behaviour.

Food-related aggregation [58] is observed in species as diverse as penguins [59], lions [60], hares [61] and even bacteria [62]. Among other advantages, formation of groups facilitates prey capture and repels predators [63]. The neurons and genes underlying group foraging behaviour have begun to be investigated in natural isolates of the nematode *C. elegans*. This species is an excellent system for the development of neurogenetic models of behaviour because the interconnections and lineages of all 302 of its neurons are known [64,65]. Furthermore, using transgenic approaches, gene expression can be restricted to specific neurons at a given stage of development to examine the precise spatial and temporal window when gene action is required [56].

Foraging in *C. elegans* appears in two forms: feeding alone when placed on a lawn of bacterial food (solitary) and feeding in groups of three to several hundred animals (aggregating) [66]. Aggregating strains also tend to show higher rates of locomotion on food, preference for thicker portions of the bacterial lawn, and more rapid habituation to ethanol [66,67]. These differences in behaviour are not found in the absence of food. Remarkably, all of these differences can be explained by variation at a single amino acid within NPR-1, a G-protein-coupled receptor of the neuropeptide Y (NPY) family [66]. As a result of the polymorphism, solitary worms contain NPR-1 receptors that are more active than those in aggregating genotypes. The *npr-1* gene appears to be required acutely during foraging, because its activation in only the adult stage of aggregating strains is sufficient to induce solitary behaviour [68].
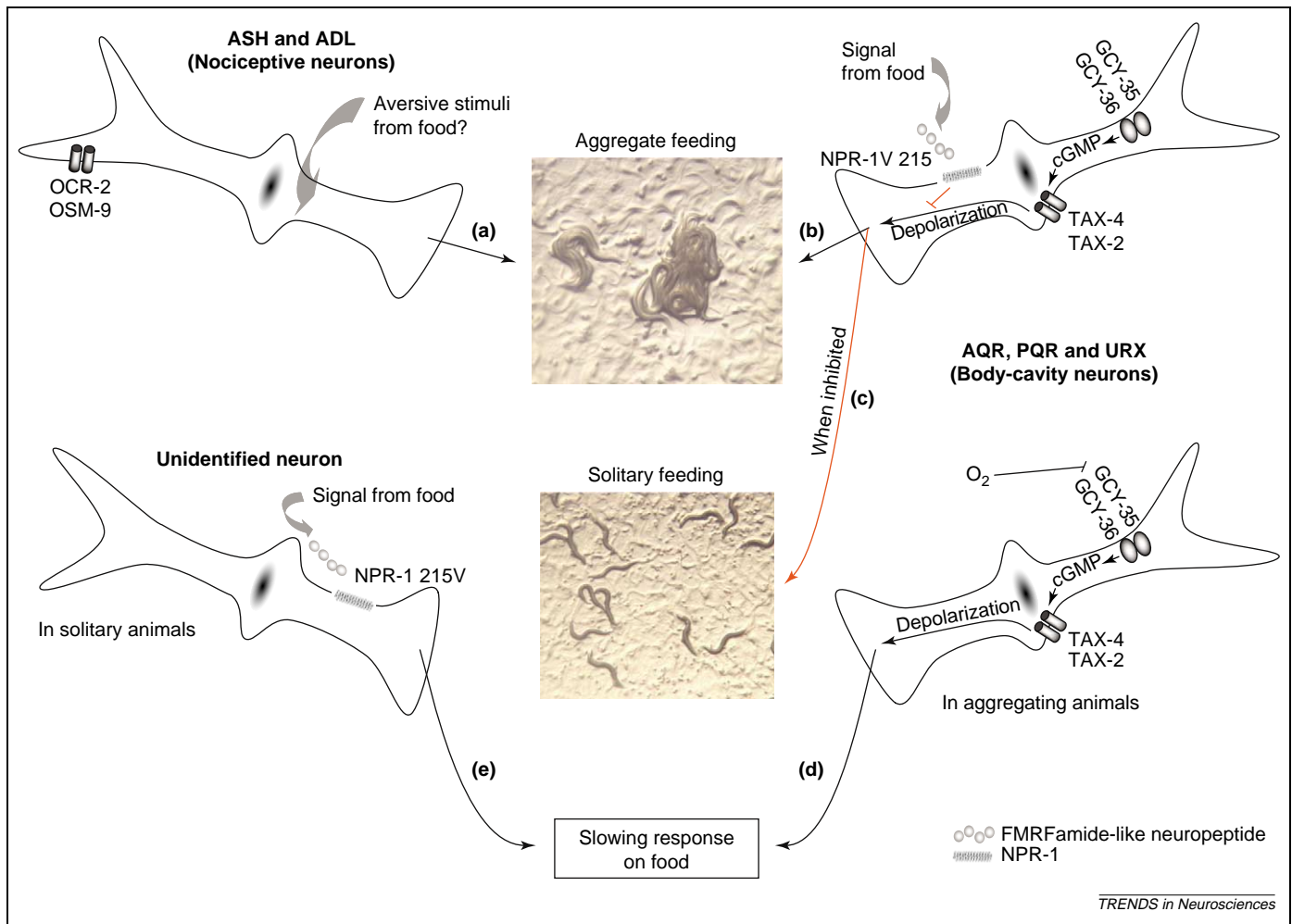
Figure 2. A neurogenetic model of food-related behaviours in *C. elegans*. Shown are events contributing to aggregate and solitary feeding, and to food-related slowing of movement. Arrows represent inductive effects, whereas blunt-ended lines indicate repression. In *C. elegans*, variation in foraging behaviour is associated with two distinct isoforms of the NPY-like receptor NPR-1. Overexpression experiments indicate that both versions of the receptor can repress aggregation [66], but in natural strains the NPR-1 215F isoform is less active and permits aggregation, whereas the NPR-1 215V isoform is more active and represses aggregation. An FMRFamide-like neuropeptide is thought to be a ligand for both isoforms of NPR-1 [79]. Images are not to scale and aggregating and solitary animals represented here are actually the same age and size. **(a)** The chemosensory neurons ASH and ADL mediate nociception and aggregation during feeding [69]. The *ocr-2* and *osm-9* genes are required for aggregation; they encode TRPV cation channel subunits that form a transduction channel linking sensory input to neuronal activity. In solitary animals, NPR-1 215V signalling suppresses this circuit, possibly by inhibiting sensitivity to nociceptive stimuli (not shown). **(b,c)** The AQR, PQR and URX body cavity neurons mediate food-dependent aggregation. Neuron depolarization and aggregation depend on a cGMP-dependent signalling pathway that employs the soluble guanylyl cyclases (sGCs) GCY-35 and GCY-36 [75] and a cGMP-gated cation channel comprising TAX-2 and TAX-4 subunits [68] (b). In solitary animals this pathway is inhibited by food-dependent signalling through NPR-1 215V (c). **(d)** Depolarization of AQR, PQR and URX neurons is also required for locomotory slowing in response to low oxygen levels in aggregating worms. This pathway also uses cGMP signalling, and moderate concentrations of oxygen inhibit sGC activity and promote relatively rapid movements. The pathway is suppressed in solitary animals by NPR-1 215V signalling [76] (not shown). **(e)** Because food-dependent slowing also occurs in solitary animals, an unidentified, oxygen-independent neural circuit involving the NPR-1 215V form of NPR-1 operates to suppress locomotion in response to food. Photographs kindly supplied by Mario de Bono.

One possible reason why *npr-1* has such diverse behavioural effects is that, similar to *fru* in *D. melanogaster* courtship, it acts through multiple neural pathways (Figure 2). One of these circuits involves the chemosensory neurons ASH and ADL, which are required for aggregation and also specialize in mediating aversive responses to noxious stimuli [69]. This connection suggests that group-feeding might be, at least in part, due to perception of aversive cues, conceivably originating from food or the animals themselves. That such a link is not intuitive is a salient instance of the power of genetics to identify regulators without bias and to bring together disparate biological domains.

Induction of solitary behaviour by NPR-1 signalling suggests that this pathway might antagonize responses of ASH and ADL to stressful stimuli. Data from other neurobiological contexts support a link between the NPY pathway and nociception, suggesting that such a system might have been adopted through natural selection in *C. elegans* to regulate foraging behaviour. For example, experiments in rats show that NPY administration in the nucleus accumbens is analgesic [70]. Likewise, mice lacking an NPY receptor experience enhanced aversion to various chemical and mechanical stimuli [71]. Whether other examples of food-related aggregation in nature are associated with nociceptive circuits remains to be determined. Interestingly, NPY has been linked to larval aggregation in *D. melanogaster* [72], suggesting that, similar to PKG and foraging locomotion, the NPY pathway could have a conserved role in mediating aggregation behaviours.

More generally, it is not surprising that stress interacts with food-related behaviours, because animals have

limited energy reserves that must be allocated differentially depending on circumstances. For example, *D. melanogaster* larvae bred in the presence of stressors such as high levels of ammonia or wasp parasitoids show reduced feeding rates and food-related movement relative to unstressed animals [73,74]. Whether energetic trade-offs exist in aggregating *C. elegans* strains with increased activity of stress-associated neurons remains to be determined. Aggregating and solitary animals should also be observed in their natural environments to determine whether they coexist and, if so, which environmental factors contribute to maintenance of the polymorphism.

In addition to input through chemosensory neurons, *npr-1* influences aggregation through a second pathway via cGMP-dependent signalling (Figure 2). Here, depolarization of neurons exposed to body fluid depends on soluble guanylyl cyclases (sGCs; these are responsible for synthesizing cGMP from GTP) and on the *tax-2* and *tax-4* cGMP-gated cation channel subunits [75]. Involvement of cGMP signalling in food-related aggregation highlights remarkable conservation of this pathway as a regulator of diverse foraging activities. In mammals, PKG has also been associated with nociceptive neuron function [26], drawing a further link between cGMP signalling and aggregation behaviour in *C. elegans*.

Other results suggest that PKG helps build plasticity into foraging behaviour. For example, Cheung and colleagues [76] found that aggregating strains of *C. elegans* fed in low oxygen levels are conditioned to prefer low oxygen levels when they are later given a choice of different oxygen concentrations. This effect is mediated by sGC activity in body-cavity neurons. Notably, because these neurons are suppressed by NPR-1 activity in solitary strains [68,76] (Figure 2), solitary animals fail to respond to depleted oxygen conditions, revealing how a change in gene expression can reconfigure sensory pathways to affect behavioural plasticity [76]. A relationship between PKG and plasticity is also observed in *D. melanogaster*, where enhanced levels of PKG in rovers versus sitters underlies reduced rates of habituation to a sucrose stimulus [24], in addition to changes in physiological measures of habituation [77]. Finally, in mammals PKG has a central role in mediating plasticity of neurotransmitter release associated with long-term potentiation [26]. Whether plasticity of other food-related behaviours, such as biting in *Aplysia*, is also regulated by cGMP–PKG-related processes could be investigated using a candidate-gene approach. An intriguing hypothesis is that coupling of cGMP signalling with both foraging and behavioural plasticity builds inherent flexibility into the mechanisms regulating food-related behaviour.

Concluding thoughts

The great diversity of food-related behaviours within nature ensures that geneticists and neurobiologists, in addition to ecologists, endocrinologists and physiologists, will have new systems to study for some time. Comparative approaches that examine both similarities and distinctions in the regulation of food-related behaviours between species will be necessary to gain an understanding of the specific neural and molecular events that underlie the evolution of food-related behaviours. In addition, it will continue to be useful to focus on a select few species to gain comprehensive knowledge of the way that genes, the environment and their interactions influence nervous system function to produce adaptive feeding behaviours. These organisms must include genetically tractable ones such as mice, *Drosophila* and *C. elegans*, but the rich wealth of neural phenotypes available in invertebrates such as molluscs and some crustaceans makes these systems very attractive for development of neurogenetic models of feeding behaviour. Future work will probably highlight the conservation of both molecular and neural principles across various feeding systems. Re-use of the cGMP signalling pathway as a regulator of multiple appetitive behaviours is a striking example of this. Other instances are emerging from nutrient-related homeostasis, where the effects of pathways such as insulin signalling and amino acid deprivation are conserved across the animal kingdom [78,20]. As new circuits affecting food-related behaviour continue to be identified, a challenge will be to determine how information from multiple pathways is consolidated and interpreted to trigger appropriate behavioural output. In all, the rich web of interactions contributing to food-related behaviour promises to be almost as fascinating as the behaviours themselves.

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