C. elegans integrates food, stress, and hunger signals to coordinate motor activity

by

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ABSTRACT

In the presence of a bacterial food source, the small nematode *C. elegans* greatly reduces its rate of locomotion. While mechanical agitation greatly stimulates the locomotion of well-fed animals on bacteria, it does not greatly stimulate the locomotion of food-deprived animals on bacteria. Thus, the competing effects of food and mechanical agitation on the animal's locomotory behavior are modulated by food deprivation. To explore how *C. elegans* modulates its locomotion we focused on determining how *C. elegans* detects bacteria, and explored the roles of biogenic amines, metabolic, and stress signaling pathways on this behavior.

We correlated specific sensory defects and the expression patterns of genes involved in sensory function, gathered by the community of *C. elegans* researchers, with abnormal responses to a bacterial food source. Our findings suggest that a soluble component of the bacterial lawn is detected by the ASH and ASE chemosensory neurons and acts to suppress locomotion, while a volatile component of the bacterial lawn is redundantly detected by multiple chemosensory neurons and acts to maintain or stimulate locomotion on bacteria.

In collaboration with Damon Clark and Aravi Samuel at Harvard University, we developed an automated locomotion tracking system that greatly improves the resolution at which we can study *C. elegans* locomotion. Using this system, we uncovered excitatory and inhibitory effects of serotonin on *C. elegans* locomotion and found that serotonin, dopamine, octopamine, and tyramine regulate the actions of one another. We also found that dopamine is required to set and maintain a precise rate of locomotion by *C. elegans*. We observed that mutants defective in dopamine signaling make crude adjustments to their speeds that result in large fluctuations in their rates of locomotion. Treatment of dopamine deficient mutants with exogenous dopamine completely rescues these locomotion defects. Removal of tyramine and octopamine together partially suppressed these defects.

We also studied how food-deprivation changes the animal's response to bacteria and mechanical agitation. We found that presumptive metabolic signaling through the *C. elegans* insulin receptor homolog *daf-2* and stress signaling through octopamine converge on the neuropeptide Y receptor homolog *npr-1* to modulate the animal's responses to food, mechanical agitation, and food-deprivation.

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Three years ago, Professor Aravi Samuel came to our laboratory to talk about technological innovations that enabled more detailed and quantitative characterization of *C. elegans* behaviors. Among these innovations was an automated locomotion tracking system. I immediately collaborated with Damon Clark, a graduate student in the Samuel lab, to build an automated locomotion tracking system of our own. This system provided a reliable way to quantify *C. elegans* locomotion and enabled new data analysis that led to many new findings presented in this thesis. I would like to thank Damon Clark and Aravi Samuel for making these last few years of my graduate career so stimulating, memorable, and productive.

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CHAPTER 1

Introduction

Integrating opposing biological drives

The acquisition of energy is a basic requirement of all living organisms and various strategies have evolved to facilitate this goal. Single-celled bacteria can detect and chemotax towards a nutrient source (BERG 1975), plants coordinate their photosynthetic activity to maximize light availability (DODD *et al.* 2005), and animals actively forage or hunt for food. At times, however, an organism's survival takes priority over the immediate acquisition of food. When conditions are crowded and nutrient levels are low, some bacteria form stress-resistant endospores to survive through unfavorable growth conditions (GROSSMAN 1995). In arid conditions plants close their stomata to prevent dehydration at the expense of carbon dioxide acquisition for photosynthesis (DODD *et al.* 2005). When imminent danger is present, animals will flee from a food-rich environment. Thus, various biological drives must be considered to ensure an organism secures an energy source and lives long enough to reproduce. These drives often exert opposing forces on the behaviors of the animal. Understanding how opposing biological drives affect *C. elegans* behavior is the goal of this thesis.

Biological drives as a simple homeostatic reflex

Biological drives can be viewed as a simple homeostatic reflex that is responsive to internal and external environmental signals. In *The Wisdom of the Body*, Cannon (1932) defines homeostasis as the ability to maintain an internally constant environment in the face of a changing external environment. Homeostasis does not reflect a stagnant but rather a dynamic system (CANNON 1932). In this context, drives are generated when the equilibrium of a system differs from a target equilibrium state or set point. The goal of these biological drives is to move the system back towards the set point.

The ability to respond to stimuli and adapt to changing environmental stimuli is critical to survival. Multiple environmental cues must often be integrated concurrently into adaptive responses. The relative simplicity of *Bacillus subtilis* greatly facilitated the discovery of a two-component phosphorelay system used by this organism to integrate food and stress signals into the decision to sporulate (HOCH 1993; GROSSMAN 1995).

Elucidation of this system greatly increased our understanding of signal transduction pathways, but the simplicity of this organism and environment makes it difficult draw parallels to our decision making process. As an organism interacts with an increasingly complex environment it must develop increasingly complex systems to integrate the additional information.

In humans, the integration of the large amount of information about our internal and external environment is achieved by an extremely complex nervous system and homeostatic mechanisms that allow us an extraordinary measure of adaptability. However, the complex nature of our environment and human drives preclude a simple study of the how competing stimuli are integrated into the decision making processes in humans. One complication is that our actions do not often obviously reflect a physiologically motivated goal and even if we make the assumption that we are the sum of our biological drives, there are many different adaptive behaviors that might lead to survival and reproductive success. To simplify the problem, we have chosen to study the effects of competing biological drives on the behavior of the small nematode *C. elegans*. *C. elegans* has a simple nervous system and a limited number of environmental factors that modulate the animal's behavior in a laboratory environment. Later we will discuss how food-deprivation modulates *C. elegans* response to food and stress.

Homeostatic regulation in humans

In the human brain, the hypothalamus governs most physiological homeostatic processes and regulates osmotic balance, body temperature, energy metabolism, reproductive processes, sleep, and emergency responses (SWANSON and SAWCHENKO 1983). Many of these processes are regulated in concert with the autonomic nervous system that allows humans to match appropriate physiological responses with the current environmental conditions (LOEWY and SPYER 1990). The autonomic nervous system consists of two branches that generally prepare the body for action or for food consumption and reproduction. The sympathetic arm of the autonomic nervous system is associated with 'fight or flight' responses that include calls to action in emergency while

the parasympathetic arm is associated with 'rest and digest' responses that include consumption of food and reproduction (CANNON 1927).

The sympathetic arm of the autonomic nervous system prepares the body for action by increasing the rate and strength of heart contractions, increasing pulmonary function, increasing sweat gland secretions, and dilating pupils. The sympathetic nervous system also suppresses food consumption and digestion related activities. The predominant neurotransmitter of the postganglionic sympathetic nervous system is norepinephrine. Norepinephrine receptors are involved in a number of related processes, including contractile effects on blood vessels, stimulatory effects on the heart, and inhibitory effects on gastrointestinal smooth muscle. Neuropeptides are also closely associated with autonomic nervous function. Neuropeptide Y is thought to be present in 90% of noradrenergic neurons, where it generally potentiates the effects of norepinephrine at target tissues far away from norepinephrine release while inhibiting the effects of norepinephrine on target tissues close to norepinephrine release (LOEWY and SPYER 1990).

The parasympathetic arm of the autonomic nervous system is complementary to the sympathetic nervous system and prepares the body for the consumption of food and reproduction. The parasympathetic nervous system generally acts in opposition to sympathetic nervous function. Among its functions, the parasympathetic nervous system slows the heart, restores normal dilation of pupils, increases gastric acid secretions and mediates arousal. Acetylcholine is the predominant neurotransmitter of the parasympathetic nervous system and has inhibitory effects on the heart and smooth muscle contraction and induces secretion from glandular tissues. Neuropeptides are also present in cholinergic neurons and include enkephalins, neurotensis, somatostatin, and substance P, which have roles in suppressing and transducing pain responses, dopamine signaling, and vasodilation (LOEWY and SPYER 1990).

The complementary actions of the sympathetic and parasympathetic nervous system allow us to maintain an internally stable environment and respond appropriately to the different demands of our changing environment. Autonomic dysfunction (dysautonomia) results when the equilibrium between the sympathetic and parasympathetic arms of the autonomic nervous system is no longer maintained. Patients

with multiple system atrophy and Parkinson's disease exhibit loss of autonomic homeostasis resulting in orthostatic hypotension (rapid drop in blood pressure), urinary urgency and incontinence, constipation, sexual dysfunction, and sialorrhea (excessive drooling) (AMINOFF and WILCOX 1971; POEWE 2006; DUBOW 2007). Parkinson's Disease is marked by deterioration of dopaminergic neurons in the brain and administration of the dopamine precursor L-DOPA (Levodopa) is currently the most effective treatment for Parkinsonian symptoms (OLANOW *et al.* 2004; TOLOSA *et al.* 2006). The relationship between reduced dopamine signaling in Parkinson's patients and loss of autonomic equilibrium suggest a role for dopamine in maintaining homeostatic balance in the autonomic nervous system.

Metabolic state and environmental stress modulate behavior

Hunger is one of the strongest influences on behavior in animals, and the drive to maintain metabolic homeostasis might be the strongest drive in all living organisms. Hunger and satiety generally describe the immediate consequences of being in a food-deprived state or well-fed, respectively. Upon consumption of a meal, satiety signals like the neuropeptide cholecystokinin that are released from the intestine into the circulatory system and eventually signal the brain to stop eating (SCHWARTZ *et al.* 2000).

In humans, hunger and satiety are intimately associated with energy metabolism, which maintains metabolic homeostasis by balancing energy intake, storage, and expenditure. Stored energy is monitored by adiposity signals that originate from fat stores and signal a positive energy balance. The adiposity signal insulin is a neuropeptide hormone that enters the brain where it acts to reduce food consumption (SCHWARTZ *et al.* 2000). Improper insulin signaling causes the metabolic disorder diabetes, which affects 7% of the population in United States and is a growing problem worldwide (CDC 2005). Leptin is another adiposity signal that reduces food intake, and mutations in the mouse *ob* gene, which encodes leptin cause obesity in mice (ZHANG *et al.* 1994). These two signals inhibit neuropeptide Y (NPY) signaling in the brain. NPY stimulates food consumption in humans (SCHWARTZ *et al.* 2000), and injection of neuropeptide Y into the brains of rats can potently stimulate food intake (STANLEY *et al.* 1986).

Stress is also a potent driver of behavioral responses that ensure the safety of the animal in adverse situations. Both organismal and cellular stress pathways can drive responses that better prepare the animal for survival under adverse conditions. In humans, the sympathetic arm of the autonomic nervous system prepares the body for action through the action of norepinephrine. As stated earlier, neuropeptide Y is co-localized with norepinephrine in a majority of noradrenergic neurons and neuropeptide Y also appears to have a role in suppressing behavioral stress responses in a number of anxiety models (HEILIG 2004). Injection of neuropeptide Y into the brains of rats has been shown to have anti-anxiety effects (HEILIG *et al.* 1993). Thus neuropeptide Y appears to have a role in both metabolic signaling and stress-related pathways.

A molecular decision making pathway in *Drosophila*

The interplay between metabolic state and stress signaling has been studied in examples of how hunger motivates animals to forage under sub-optimal or even dangerous conditions (KACELNIK 1996). The genes involved in this decision are now being identified in animal systems. The effects of metabolic state on foraging under aversive conditions have been recently studied in *Drosophila melanogaster* (LINGO *et al.* 2007). Lingo and colleagues (2007) find that at cold temperatures well-fed *Drosophila* larvae are discouraged from foraging for food. These temperatures, however, do not discourage food-deprived *Drosophila* larvae from foraging to the same extent (LINGO *et al.* 2007). They also found that this response was modulated by the *Drosophila* neuropeptide Y like signaling molecule NPF (LINGO *et al.* 2007). Overexpression of NPF resulted in increased foraging of well-fed larvae under cold conditions while RNA mediated interference of the neuropeptide Y-like receptor *npfr1* (neuropeptide F receptor-1) resulted in reduced foraging of food-deprived larvae under cold conditions (LINGO *et al.* 2007). These and other findings suggest that NPY-like signaling in flies might be involved in a motivational food response in which the effects of stress and hunger compete in the decision making process (WU *et al.* 2005; LINGO *et al.* 2007). This study of simple behavior begins to provide a context within which to study the molecular mechanisms by which competing stimuli are integrated into a decisive action.

The study of competing biological drives in *C. elegans*

The small free living nematode *C. elegans* is well suited for studying how competing drives like hunger and stress affect behavior. *C. elegans* respond to a wide range of stimuli (BARGMANN and MORI 1997; DRISCOLL and KAPLAN 1997), and exhibit robust behavioral and developmental responses to food and stress (AVERY and THOMAS 1997; RIDDLE and ALBERT 1997). These animals also exhibit behavioral plasticity (JORGENSEN and RANKIN 1997), have a simple yet highly conserved nervous system (BARGMANN 1998), and exhibit behavior in which the competing effects of food and mechanical agitation are modulated by food-deprivation. The self-fertilizing *C. elegans* hermaphrodite has a short life cycle of only three days and can produce hundreds of progeny in that time (RIDDLE *et al.* 1997). Genetic analysis of *C. elegans* is greatly facilitated by the ability to perform forward genetic screens, gene knockouts, RNA interference, transposon mutagenesis, and the ability to create transgenic strains (JANSEN *et al.* 1997; JOHNSEN and BAILLIE 1997). *C. elegans* also has a transparent body, such that expression of genes using appropriate reporters can be determined in the intact living animal (RIDDLE *et al.* 1997). The entire invariant cell lineage of the worm has been determined, which greatly improves the resolution with which we can study the development of the animal and allows us to ascribe biological functions to morphologically identifiable cells (SULSTON and HORVITZ 1977).

***C. elegans* responds to a wide range of environmental stimuli**

C. elegans detects a wide range of environmental stimuli and has a relatively simple nervous system that integrates the cues that modulate behavioral outputs. These cues include volatile and soluble chemicals, temperature, and various forms of mechanical stimuli (WARD 1973; HEDGECOCK and RUSSELL 1975; CULOTTI and RUSSELL 1978; CHALFIE *et al.* 1985; BARGMANN *et al.* 1993; KAPLAN and HORVITZ 1993). *C. elegans* also responds to many of these stimuli in a graded fashion, exhibiting chemotaxis towards certain volatile and soluble chemicals at low concentrations but

avoiding them at higher concentrations (WARD 1973; CULOTTI and RUSSELL 1978; BARGMANN *et al.* 1993). Thus *C. elegans* is able to completely change its behavioral response based not only on the presence or absence of an environmental cue but also on the strength of that cue.

Laser ablation of neurons has been used to assign specific functions to many neurons of the *C. elegans* nervous system (SULSTON and WHITE 1980; CHALFIE *et al.* 1985). The ciliated processes of eight types of sensory neurons are present in the bilateral amphid channels of the animal, which is open to the external environment (WARD *et al.* 1975). The amphid neurons are responsible for the detection of soluble chemicals including salts, cAMP, biotin, lysine, and others (BARGMANN *et al.* 1990; BARGMANN and HORVITZ 1991a; BARGMANN and HORVITZ 1991b; KAPLAN and HORVITZ 1993). A second set of sensory neurons with flattened and branched morphology have been associated with the detection of volatile odorants including diacetyl, pyrazine, thiazole, benzaldehyde, butanone, and isomyl alcohol (BARGMANN *et al.* 1993).

C. elegans behaviors are extensively modulated by a bacterial food source

In the laboratory, *C. elegans* are grown on a bacterial food source that elicits a number of food-dependent behaviors including feeding (AVERY and HORVITZ 1990), egg-laying (HORVITZ *et al.* 1982), and reduction of locomotion (SAWIN *et al.* 2000). In the absence of a food source and in crowded conditions, early *C. elegans* larvae will enter a stress-resistant dauer state, entry into which is suppressed by a bacterial food source (GOLDEN and RIDDLE 1984). Until recently, a bacterial food source was thought about simply as providing a rewarding food stimulus for *C. elegans*. Studies of *C. elegans* longevity, food quality, and bacterial pathogenicity began to suggest all bacterial food sources are not the same and a bacterial lawn can also harm animals (DE BONO *et al.* 2002; ZHANG *et al.* 2005). Our study of the relationship between sensory function and the response of *C. elegans* to a bacterial lawn suggests that *C. elegans* uses a common set of neurons to detect a complex set of bacterial cues that can both stimulate and repress *C. elegans* locomotion (Chapter 2). Our findings also suggest dauer signals and food

might be detected in a similar fashion. We propose a simple model for the integration of dauer pheromone and food signals that is consistent with data collected by the *C. elegans* community (Chapter 2).

C. elegans exhibits behavioral plasticity

C. elegans exhibits a number of other behaviors that are characteristic of behavioral plasticity, including sensory adaptation, habituation, sensitization, and associative learning. *C. elegans* exhibits chemotaxis towards or avoidance of many volatile and soluble chemical signals. These responses are reduced after extended exposure to these chemicals (WARD 1973; DUSENBERY 1980; COLBERT and BARGMANN 1995). Animals are also able to acclimate to novel temperatures that they once avoided (HEDGECOCK and RUSSELL 1975). Additionally, drug sensitivities are altered upon extended exposure to drugs. We found that animals grown on high concentrations of exogenous serotonin (20 mM) become resistant to immobilization by exogenous serotonin.

Habituation and sensitization are two aspects of simple non-associative learning and memory in which the responses of an animal are changed after repeated exposure to a single stimulus. Habituation differs from sensory adaptation in that it can be built up by a series of training sessions, recovery rate depends on intervals of training, and animals can be dishabituated by exposure to a noxious stimulus (GROVES and THOMPSON 1970). After a period of receiving mechanical stimuli, *C. elegans* show reduced responses to the same mechanical stimulus and sensitivity can be restored by electrical shock (RANKIN *et al.* 1990). Conversely, *C. elegans* trained with a single mechanical stimulus followed by a series of stronger stimuli become sensitized to future single stimulus events (RANKIN *et al.* 1990).

C. elegans has also been shown to exhibit associative learning. In an ionic gradient where unconditioned wild-type *C. elegans* exhibits no ionic preference for chloride or sodium ions, conditioning worms first with food and either paired ion increased the preference of animals for the food-paired ion. Conversely, conditioning

worms first with garlic and either paired ion decreased the preference of animals away from the garlic-paired ion (WEN *et al.* 1997).

The simple yet highly conserved *C. elegans* nervous system

The simple and complex behaviors of *C. elegans* are generated by a nervous system of only 302 neurons, the connectivity of which was determined from serial section electron micrographs (WHITE *et al.* 1986). The nerve ring is a region of high neuronal process density that might function as a central information exchange in the nervous system of *C. elegans*. The structure and connectivity of the neurons in the *C. elegans* nervous system are reminiscent of the postganglionic neurons of the autonomic nervous system that form synapses en passant at multiple places along target tissues, contain non-traditional pre-synaptic specializations, and have almost no post-synaptic specialization (WHITE *et al.* 1986; IVERSEN *et al.* 2000). These connections in humans have been speculated to facilitate action at a distance exerting a more diffuse effect on targets. The neuroanatomy of *C. elegans* suggests that humoral signaling might play an important role in the small nematode, because diffusion could easily transport molecules throughout much of the animal.

Despite the relative anatomical simplicity of the *C. elegans* nervous system, the *C. elegans* genome encodes voltage-gated calcium and potassium channels, ligand gated ion channels, classic neurotransmitter synthesis and degradation enzymes, neurotransmitter transporters, neuropeptides and neuropeptide receptors, G proteins and G protein coupled receptors, mechanosensory proteins, cyclic nucleotide signaling pathways, and others (BARGMANN 1998). Serotonin, dopamine, and the invertebrate noradrenergic analog octopamine are also present in *C. elegans* (SULSTON *et al.* 1975; HORVITZ *et al.* 1982) as are insulin- (KIMURA *et al.* 1997) and neuropeptide Y-like receptors (DE BONO and BARGMANN 1998) involved in human metabolic processes.

Biogenic amines in human and *C. elegans* behaviors

In humans, the majority of serotonin is present in the gut and appears to be related to digestive function (DE PONTI 2004). However, serotonin also has neuroactive function and has been shown to affect multiple brain functions, including appetite, mood, sleep, and motor function (FRAZER and HENSLER 1999). Selective serotonin reuptake transporter inhibitors (SSRIs) are an important class of drugs used in the treatment of depression and include flouxetine (Prozac) (NIERENBERG *et al.* 2000). Serotonin is more generally thought to have a role in satiety. The majority of serotonin receptor agonists and SSRIs decrease food intake in humans, as does peripheral administration of serotonin in rats (SIMANSKY 1996). In *C. elegans*, exogenous serotonin stimulates food-related behaviors, including pharyngeal pumping, egg-laying, and suppression of locomotion (HORVITZ *et al.* 1982). The relationship between serotonin and these behaviors has also been shown in the context of endogenous serotonin signaling (TRENT *et al.* 1983; SZE *et al.* 2000; RANGANATHAN *et al.* 2001). Thus, the relationship between serotonin and food appears to be conserved between worms and humans.

Earlier I mentioned that norepinephrine is associated with 'fight or flight' responses in emergency situations. Octopamine is the invertebrate analog of norepinephrine and is thought to modulate a majority of physiological processes in insects (ROEDER 1999). In insects, octopamine functions as a stress hormone triggered by starvation and other environmental stress (DAVENPORT 1984). In numerous *C. elegans* behaviors serotonin and octopamine act antagonistically to each other. While exogenous serotonin stimulates food-related behaviors like pharyngeal pumping and egg-laying, exogenous octopamine inhibits pharyngeal pumping and egg-laying (HORVITZ *et al.* 1982). Thus serotonin and octopamine appear to have opposing functions in the stimulation and inhibition of food and reproductive responses, much like the parasympathetic and sympathetic arms of the human autonomic nervous system. Tyramine is the biosynthetic precursor to octopamine and has also been shown to have biological activity in the suppression of foraging behaviors in *C. elegans* (ALKEMA *et al.* 2005).

Dopamine signaling modulates many mammalian neurobiological processes, including motor control, learning, and motivation (WISE 2004; SCHULTZ 2007). Abnormal dopamine function has been associated with a variety of disorders, including

Parkinson's Disease, attention-deficit hyperactivity disorder (ADHD), and addiction (SOLANTO 2002; WISE 2004; HORNYKIEWICZ 2006). Reduced dopamine signaling in Parkinson's patients leads to motor coordination defects and loss of autonomic homeostasis with high frequency (AMINOFF and WILCOX 1971; GERMAN *et al.* 1992; POEWE 2006; DUBOW 2007). Dopamine is also involved in reward behavior and is released when animals encounter an improvement in conditions, which is usually a food reward in experimental contexts (SAPER *et al.* 2002; WISE 2004; SCHULTZ 2007).

In *C. elegans* dopamine signaling is involved in the detection of a bacterial lawn (SAWIN *et al.* 2000) and in habituation to mechanical stimuli (SANYAL *et al.* 2004). A bacterial food source suppresses *C. elegans* locomotion and mutants with defects in dopamine signaling reduce their locomotion less in response to bacteria (SAWIN *et al.* 2000). Tapping the side of the culture plate containing *C. elegans* animals induces animals to initiate backward locomotion called a reversal (RANKIN *et al.* 1990). After the administration of repeated plate taps, animals become habituated to this form of mechanical stimulus and initiate reversals less frequently in response to plate tap (RANKIN *et al.* 1990). Mutants with attenuated levels of dopamine exhibit less habituation than wild-type animals (SANYAL *et al.* 2004).

The roles of neuropeptides in *C. elegans* behaviors

The *C. elegans* genome contains a large number of predicted neuropeptides (HUSSON *et al.* 2007) and neuropeptide receptors that have been shown to have roles in regulation of feeding behavior (DE BONO and BARGMANN 1998), lifespan (KENYON *et al.* 1993), aerotaxis (CHEUNG *et al.* 2005), dauer formation (KIMURA *et al.* 1997), egg-laying (WAGGONER *et al.* 2000), and coordination (NELSON *et al.* 1998). The two most extensively characterized neuropeptide receptors are the insulin receptor homolog *daf-2* and the neuropeptide Y/FF-like receptor *npr-1*.

The insulin receptor homolog *daf-2* is involved in a number of metabolically regulated processes. As mentioned earlier, the absence of food stimulates early *C. elegans* larvae to enter a stress resistant dauer state that is optimized for long term survival without food (CASSADA and RUSSELL 1975). Mutation of *daf-2* results in the

formation of constitutive dauers consistent with a role for this insulin receptor homolog in the transduction of metabolic signals that inhibit dauer formation in *C. elegans* (RIDDLE *et al.* 1981; KIMURA *et al.* 1997). *daf-2* mutants also have increased fat storage (OGG *et al.* 1997), increased longevity (KENYON *et al.* 1993), and are resistant to high temperature stress (LITHGOW *et al.* 1995), all typical of dauer larvae.

npr-1-dependent signaling in *C. elegans* is also involved in many food-dependent behaviors. Mutations in *npr-1* lead to hyperactivity on bacteria and social feeding, a behavior in which animals aggregate together on a bacterial lawn (DE BONO and BARGMANN 1998). Social feeding behavior was inhibited by high concentrations of oxygen (CHEUNG *et al.* 2005). Oxygen pressure preference leads to movement guided by oxygen gradients called aerotaxis, which was observed in wild-type animals in the absence of bacteria (CHANG *et al.* 2006). In the presence of a bacterial lawn, aerotaxis behavior was inhibited (CHANG *et al.* 2006). *npr-1* mutants also exhibit oxygen pressure preference but this preference was not as strongly inhibited by the presence of bacteria (CHANG *et al.* 2006). Since wild-type animals aggregate at high-population density, move faster, and are sensitive to oxygen gradients in the absence of bacteria, but not in the presence of bacteria, collectively these results might suggest that *npr-1* mutants might have altered sensitivities to bacteria.

Modulation of locomotion by acute food deprivation

Beth Sawin, a previous Horvitz laboratory graduate student, first described the modulated behavior that is the foundation for my thesis work. She found that upon entering a bacterial food source well-fed worms reduce their rate of locomotion a small amount, in what she called the basal slowing response. After acute food-deprivation animals entering bacteria reduce their rate of locomotion substantially more, in what she called the enhanced slowing response. Thus bacteria elicit a different response from animals that are well-fed than from animals that are food-deprived.

In a series of genetic, pharmacologic, and neuronal ablation experiments, Beth Sawin showed that the basal slowing response relied on the ADE, PDE, and CEP dopaminergic mechanosensory neurons while the enhanced slowing response partially

depended on the neurotransmitter serotonin (SAWIN *et al.* 2000). The finding that mutations in the serotonin gated chloride channel *mod-1* and the serotonin reuptake transporter *mod-5* both affected the locomotion of animals further supported a role for serotonin in the modulation of *C. elegans* locomotion (SAWIN 1996; RANGANATHAN *et al.* 2000). Subsequent characterization of serotonin signaling mutants *mod-1* and *mod-5* mutants suggested that serotonin was specifically involved in the enhanced slowing response and that dopamine and serotonin functioned in a non-overlapping manner with respect to these two responses (RANGANATHAN *et al.* 2000; RANGANATHAN *et al.* 2001).

Since these initial studies, a mutant that specifically lacked serotonin (SZE *et al.* 2000) and a mutant that lacked the invertebrate norepinephrine analog octopamine and related biogenic amine tyramine (ALKEMA *et al.* 2005) have been isolated. The role of octopamine in the modulation of *C. elegans* locomotion was not previously explored with respect to the modulation of *C. elegans* locomotion rate. We performed genetic epistasis experiments between mutants that specifically lack dopamine, serotonin, tyramine, and octopamine uncovered a balance of excitatory and inhibitory interactions between these neurotransmitters that modulate *C. elegans* locomotion (Chapter 3). These regulatory interactions support the idea that biogenic amines act like a simple autonomic nervous system to modulate food and stress related activity in *C. elegans*.

The search for genes involved in the modulation of *C. elegans* locomotion by food deprivation

To identify genes that reduce the locomotion of animals on bacteria after food deprivation, Beth Sawin performed a genetic screen for mutants that moved faster than wild-type animals on a bacterial lawn after food-deprivation (SAWIN *et al.* 2000). These Mod mutants proved difficult to clone and we hope to simplify the cloning process by identifying additional mutants in a conceptually identical screen performed in a sensitized genetic background. Mutations in the *C. elegans* serotonin reuptake transporter *mod-5* cause animals to exhibit a serotonin dependent paralysis upon entering a bacterial lawn in the food-deprived state (SAWIN *et al.* 2000; RANGANATHAN *et al.* 2001). Using a *mod-5*

mutant background we screened for mutants that failed to become paralyzed upon entering a bacterial lawn in the food-deprived state (Appendix B).

My initial interest in studying this modulated behavior in *C. elegans* was to explore the molecular and cellular mechanism by which *C. elegans* generates and stores information about its food-deprived state and uses this information to modulate its behavior. Inspired by human hunger and satiety signaling mechanisms, we proposed that animals defective in satiety signaling might exhibit greater slowing not only after food-deprivation but also in the well-fed state. In a complementary screen, we looked for mutants that exhibited food-dependent paralysis, typical of food-deprived *mod-5* mutants, but in the well-fed state (Appendix B).

An automated locomotion tracking system identifies a new role for dopamine in the coordination of *C. elegans* locomotion and redefines the Mod Assay

In collaboration with Damon Clark in the laboratory of Aravi Samuel in the Harvard Physics Department, we created an automated locomotion tracking system to improve data collection. Specifically we hoped to increase the rate at which data could be collected and to remove human quantification of locomotion. After completion we found that our tracking system had the added advantage of increased temporal and spatial resolution, removal of environmental variables that affect our assay, and new types of data analysis (Appendix A).

Our automated tracking system allowed us to study and quantify different aspects of *C. elegans* locomotion that had not previously been studied. Using this system we explored a simple observation made about the locomotion of animals lacking dopamine. Upon scoring the locomotion of animals manually, we found that the mutants lacking dopamine appeared to move both faster and slower than wild-type animals on a bacterial food source. Since our automated tracking system maintains the integrity of individual tracks we were able to study how individual animals changed their locomotion rates over time. In our analysis we found that dopamine is required to set and maintain a precise rate of locomotion in *C. elegans* (Chapter 4).

Our tracking system also enabled us to study the kinetics of food deprivation and the duration of each response, the results of which fundamentally changed the biological context with which we think now think about this behavior. These experiments show that the Mod Assay is one in which the competing effects of mechanical agitation and food on activity levels are modulated by acute food deprivation (Chapter 5).

A model for decision making in *C. elegans*

The locomotion of well-fed undisturbed animals is greatly suppressed in the presence of bacteria. In the Mod Assay, transferring well-fed animals to assay plates causes transiently elevated locomotion in the presence of bacteria. Thus when animals are well-fed, mechanical agitation has a temporarily greater effect on the locomotion of animals than bacteria. Transferring food-deprived animals to assay plates does not cause elevated locomotion to the same extent. In this case the effect of bacteria has a greater effect on the locomotion of animals than mechanical agitation. Acute food-deprivation causes this shift in behavior.

Using the Mod Assay we began our exploration into the decision making process of the worm. In the final chapter of this thesis we describe how a conserved neuropeptide signaling pathway appears to integrate metabolic and stress signals that modulate *C. elegans* locomotory behavior (Chapter 6).

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CHAPTER 2

The altered locomotion of sensory mutants on a bacterial food source suggest that bacterial cues can both inhibit and stimulate *C. elegans* locomotion

Summary

A bacterial food source dramatically modulates the behavior and development of *C. elegans*. In our assay of modulated locomotion, food-deprived animals reduce their rate of locomotion dramatically upon encountering bacteria. Mutants with a specific defect in the detection of soluble chemicals reduce their rate of locomotion less than wild-type animals on bacteria. In contrast, mutants with broad defects in chemosensation reduce their locomotion more than wild-type animals upon contact with bacteria. Reduced slowing on bacteria correlates with mutations in genes that are expressed in a limited number of chemosensory neurons, while increased slowing on bacteria correlates with mutations in genes that are expressed broadly in sensory neurons. Our results suggest that soluble components of a bacterial lawn are detected by the ASE and ASH neurons and inhibit *C. elegans* locomotion and that volatile components of a bacterial lawn are detected by many sensory neurons and sustain *C. elegans* locomotion. The inability to sustain locomotion on a bacterial lawn appears to mask the reduced ability to slow in response to a bacterial lawn in the context of sensory function. Individual sensory neurons also appear to have common functions in detecting stimuli that have opposing effects on dauer formation.

Introduction

Many *C. elegans* behaviors are modulated by a bacterial food source including feeding (AVERY and HORVITZ 1990), egg-laying (HORVITZ *et al.* 1982), and locomotion rate (SAWIN *et al.* 2000). In the absence of bacteria, *C. elegans* hermaphrodites move at a rapid rate across the agar surface of a culture plate. In the Mod Assay, well-fed animals exhibit slightly reduced locomotion upon entering a bacterial lawn, while food-deprived animals exhibit increased slowing upon entering the same bacterial lawn. The reduced locomotory rates of well-fed and food-deprived animals on bacteria were named the basal and enhanced slowing responses respectively (SAWIN *et al.* 2000). The putative mechanosensory ADE, PDE, and CEP neurons are the only dopamine positive cells in

C. elegans (SULSTON *et al.* 1975), and surgical ablation of these neurons eliminates the basal slowing response (SAWIN *et al.* 2000). Thus, it was hypothesized that mechanical detection of a bacterial lawn by dopamine positive neurons contributed to the basal slowing response (SAWIN *et al.* 2000). Mutants lacking dopamine still exhibited enhanced slowing, which suggested that food-deprived animals detected bacteria in a dopamine-independent fashion (SAWIN *et al.* 2000). Since *C. elegans* can detect a wide range of soluble and volatile chemicals, it had long been hypothesized that chemosensation likely plays a role in the detection of bacteria (WARD 1973; CULOTTI and RUSSELL 1978; BARGMANN *et al.* 1993).

One example in which chemosensory neurons appear to play a role in the detection of bacteria is in the inhibition of dauer formation (BARGMANN and HORVITZ 1991b). The dauer stage is an alternate stress-resistant larval stage that animals enter when conditions are not favorable for growth and reproduction (CASSADA and RUSSELL 1975). High concentrations of dauer pheromone, produced by *C .elegans*, indicates crowded conditions and stimulates dauer formation, while food signals indicate favorable growth conditions and inhibit dauer formation (GOLDEN and RIDDLE 1982; GOLDEN and RIDDLE 1984a). The balance between these two opposing signals affects the decision between entering dauer stage and continuing development into a reproductive adult (GOLDEN and RIDDLE 1984b). Ablation of the ADF, ASG, ASI, and ASJ chemosensory neurons results in the constitutive entry of animals into dauer development; therefore these neurons might be involved in the detection of food signals that inhibit dauer formation (BARGMANN and HORVITZ 1991b).

The chemosensory defects of large number of mutants have been identified and the expression pattern of genes mutated in these strains have been determined (BARGMANN and MORI 1997). Additionally, neuronal ablation experiments have assigned the detection of various soluble and volatile odorants to specific sensory neurons in *C. elegans* (BARGMANN *et al.* 1993). Our survey relied on these chemosensory and expression data collected by the community of *C. elegans* researchers.

We first looked at the enhanced slowing responses of sensory mutants and attempted to correlate enhanced slowing defects with specific chemosensory defects. Specifically, we identified groups of mutants with similar locomotion defects and then

looked for chemosensory defects that they had in common. We next attempted to identify neurons that are involved in the food-dependent modulation of locomotion by correlating the neuronal expression of each gene with a specific locomotion defect. Specifically, we looked for overlapping neuronal expression of chemosensory genes that when mutated caused similar locomotion defects. In our analysis we make the assumption that if a gene is expressed in a neuron, mutation in that gene results in the loss of that neuron's function. While this criterion might not be met in every case, we do not base our conclusions on any single gene's expression but rather on the overlapping expression of multiple genes.

We looked at a panel of mutants with defects in the detection of both soluble and volatile chemicals. Chemotaxis data existed for multiple mutants with respect to the avoidance of, and chemotaxis towards, sodium chloride and the detection of volatile odorants isoamyl alcohol, pyrazine, diacetyl, trimethyl thiazole, and benzaldehyde (WARD 1973; BARGMANN and HORVITZ 1991a; BARGMANN *et al.* 1993). We also looked at mutants with defects in other sensory processes, including thermotaxis, mechanosensation, and nose touch avoidance (HEDGECOCK and RUSSELL 1975; CHALFIE *et al.* 1985; KAPLAN and HORVITZ 1993). In our locomotion assays, we attempted to use the same alleles and strains used in chemotaxis assays.

Since most genes that are involved in chemosensory function are expressed in chemosensory neurons, we focused our attention on the expression of genes in the ADF, ADL, ASE, ASG, ASH, ASI, ASJ, and ASK chemosensory neurons of the amphid sensillum, PHA and PHB phasmid neurons, and AWA, AWB, and AWC neurons involved in the detection of volatile odorants (WARD *et al.* 1975; PERKINS *et al.* 1986; BARGMANN *et al.* 1993; HILLIARD *et al.* 2002). We also looked at the ADE and CEP dopaminergic neurons, the AQR and PQR neurons with proposed function in monitoring components of body fluid, and the AFD thermosensory neuron (SULSTON *et al.* 1975; PERKINS *et al.* 1986; COATES and DE BONO 2002).

Since chemosensation has been implicated in the detection of a bacterial food source, which suppresses *C. elegans* locomotion, we expected to identify only chemosensory mutants that exhibited reduced enhanced slowing. Our results differed

strikingly from our expectations and suggested that there are multiple bacterial signals that have opposing effects on *C. elegans* locomotion.

Results and Discussion

Defects in salt detection and gene expression in the ASE and ASH sensory neurons correlate with reduced enhanced slowing

che-1(p674) (CHEmotaxis defective) and *osm-8(n1518)* (OSMotic avoidance defective) mutants both exhibit reduced enhanced slowing (Figure 2.1). *che-1* encodes a zinc-finger containing transcription factor that when mutated results in defects in both the avoidance of high concentrations of sodium chloride and chemotaxis towards lower concentrations of sodium chloride (UCHIDA *et al.* 2003). *che-1(p674)* mutants are normal for the detection of all volatile odorants mentioned in the introduction (BARGMANN *et al.* 1993). *osm-8* is an unpublished gene that also has a defect in detecting high concentrations of sodium chloride (Jim Thomas, personal communication). Thus the detection of salt is impaired in both *che-1* and *osm-8* mutants (Table 2.1).

che-1 is expressed in only the ASH and ASE sensory neurons (UCHIDA *et al.* 2003). *osm-8* is expressed in only the ASH neurons (Jim Thomas, personal communication). The ASE neuron plays a role in the detection of soluble molecules like sodium chloride, cAMP, biotin, and lysine (BARGMANN and HORVITZ 1991a) and the ASH neuron is involved in detection of high osmolarity, high concentrations of benzaldehyde, and octanol (TROEMEL *et al.* 1995). Since *che-1(p674)* and *osm-8(n1518)* mutants each exhibit reduced enhanced slowing, fail to detect soluble chemicals, and are both expressed in the ASH neurons, we hypothesize that soluble bacterial factors are likely detected by the ASH, and possibly ASE neurons, and inhibit locomotion in the enhanced slowing response (Table 2.2). Laser ablation of these neurons would more decisively define their roles in the enhanced slowing response.

Mutants defective in only the avoidance of high concentrations of sodium chloride modulate their locomotion normally

Both *che-12(e1812)* and *osm-3(p802)* mutants have normal enhanced slowing responses (Figure 2.2). Mutations in *che-12* result in defects in secretion of matrix material that forms the pore of the amphid sensillum that exposes the amphid sensory neurons to the external environment. *che-12* mutants are defective in the avoidance of high concentrations of sodium chloride but are normal for chemotaxis towards sodium chloride and all volatile odorants mentioned (PERKINS *et al.* 1986; BARGMANN *et al.* 1993). Mutation in the anterograde motor kinesin gene *osm-3* leads to the same chemosensory defects but by a different mechanism (CULOTTI and RUSSELL 1978; PERKINS *et al.* 1986; BARGMANN *et al.* 1993; SHAKIR *et al.* 1993). Thus, it appears that a defect in the avoidance of high concentrations of salt alone does not correlate with a modulation of locomotion defect (Table 2.1). These data suggest that the ability to chemotax toward soluble molecules, and not osmotic avoidance of soluble molecules, correlates with the capacity to detect bacterial cues that reduce the locomotion of *C. elegans*.

Broad defects in sensory function and common expression in four sensory neurons correlate with increased enhanced slowing

che-3(e1124), *osm-1(p808)*, and *osm-6(p811)* mutants have increased enhanced slowing (Figure 2.3). *che-3* encodes a cytoplasmic dynein heavy chain that is necessary for the development and maintenance of ciliated processes of sensory neurons (PERKINS *et al.* 1986; SIGNOR *et al.* 1999; WICKS *et al.* 2000). CHE-3 motor proteins are involved in the retrograde intraflagellar transport of raft particles OSM-1 and OSM-6 (SIGNOR *et al.* 1999). *osm-1* and *osm-6* are also required for the proper formation of these ciliated processes (PERKINS *et al.* 1986). *che-3*, *osm-1*, and *osm-6* mutants have a reduced ability to detect sodium chloride and all volatile odorants tested, which included isoamyl alcohol, pyrazine, diacetyl, trimethyl thiozole, benzaldehyde and butanone (CULOTTI and RUSSELL 1978; PERKINS *et al.* 1986; BARGMANN *et al.* 1993).

Both *tax-2(p691)* and *tax-4(p678)* mutants (chemoTAXis abnormal) also exhibit a greater enhanced slowing response (Figure 2.3). *tax-2* and *tax-4* encode cyclic nucleotide gated ion channels involved in chemosensation (COBURN and BARGMANN 1996; KOMATSU *et al.* 1996). Mutations *tax-2* and *tax-4* cause broad sensory defects that completely overlap with those of *che-3*, *osm-1*, and *osm-6* mutants in all conditions tested. *tax-2* and *tax-4* mutants have reduced chemotaxis to sodium chloride and all volatile odorants tested (BARGMANN *et al.* 1993; KOMATSU *et al.* 1996; COBURN *et al.* 1998).

odr-4(n2144) (ODoRant response abnormal) mutants are defective specifically in the detection of diacetyl, trimethyl thiozole, and benzaldehyde and exhibit an increased enhanced slowing response (BARGMANN *et al.* 1993; DWYER *et al.* 1998) (Figure 2.3). *odr-4* encodes a novel protein that is implicated in the recruitment of seven-transmembrane receptors to the plasma membrane (DWYER *et al.* 1998). While mutation of *odr-4* does not cause global defects in olfaction, these mutants have a defect in the detection of trimethylthiazole, which is redundantly detected by a number of olfactory neurons. Strangely, *odr-4* mutants can still chemotax towards odorants that are detected by the individual neurons that redundantly detect trimethylthiazole (BARGMANN *et al.* 1993). One possibility is that ODR-4 is required to recruit receptors involved specifically in the detection of trimethylthiozole to the plasma membrane.

Studies by Cori Bargmann, a previous post-doc in the Horvitz lab, showed that the winged ciliated neurons AWA and AWC are responsible for detecting the majority of volatile chemical odorants tested on *C. elegans* (BARGMANN *et al.* 1993). She showed that laser ablation of the AWA neuron results in greatly impaired detection of diacetyl and pyrazine, while laser ablation of AWC neurons results in impaired detection of benzaldehyde, butanone, and isoamy alcohol. Ablation of both AWA and AWC neurons together result in reduced detection of all of these volatile odorants and trimethylthiazole, which is detected by both AWA and AWC neurons (BARGMANN *et al.* 1993). The ASH sensory neuron has also been shown to be involved in the detection of benzaldehyde and thus the AWC and ASH have redundant functions in the detection of this chemical (TROEMEL *et al.* 1995).

che-3, *osm-1*, *osm-6*, *tax-2*, *tax-4*, and *odr-4* are all expressed in the ASI, ASJ, ASK, and ASG neurons of the amphid sensillum, which have been shown to be involved in chemosensation. (COBURN and BARGMANN 1996; KOMATSU *et al.* 1996; COLLET *et al.* 1998; WICKS *et al.* 2000; MCKAY *et al.* 2003) (Table 2.2). Mutations in *che-3, osm-1,* and *osm-6* result in defects in detection of all volatile odorants but are not reported to be expressed in the AWA or AWC neurons that have been shown to detect volatile chemicals (BARGMANN *et al.* 1993; COLLET *et al.* 1998; WICKS *et al.* 2000; MCKAY *et al.* 2003) (Table 2.2). These expression data were particularly unexpected because *che-3, osm-1,* and *osm-6* are involved in the formation of ciliated processes and the AWA and AWC neurons are some of the most heavily ciliated neurons in *C. elegans*.

One possibility is that expression of these proteins or reporter gene is too weak to detect. A second possibility is that while these genes are not expressed in these neurons, the functions of AWA and AWC neurons are affected indirectly by mutations in these genes. For instance, these genes might act in neurons that modulate AWA or AWC function, transduce AWA or AWC signals, or are required for the proper development of the AWA and AWC neurons. Since the expression patterns of *che-3*, *osm-1*, and *osm-6* were determined by GFP reporter gene expression (COLLET *et al.* 1998; WICKS *et al.* 2000; MCKAY *et al.* 2003), a third possibility is that GFP reporter gene expression itself causes abnormal neuronal process development, which has been reported to occur, thus precluding visualization of these reporters (Niels Ringstad, personal communication).

che-3, osm-1, osm-6, tax-2, and *tax-4* mutants fail to detect all odorants detected by both AWA and AWC neurons and exhibit reduced locomotion on a bacterial lawn (BARGMANN *et al.* 1993; KOMATSU *et al.* 1996). While *odr-4* mutants can still detect some volatile odorants, they fail to detect trimethylthiazole, which is detected by the AWA and AWC neurons, and also exhibit reduced locomotion. These data suggest that a component of the bacterial lawn is required to maintain locomotion upon entering a bacterial lawn and that this bacterial cue is likely volatile. Since most of the mutants that exhibit more limited defects in olfaction were not tested for salt chemotaxis defects, we cannot eliminate the possibility that a defect in detecting salts and any single odorant together might lead also lead to reduced locomotion on bacteria.

Interestingly the broad defects of *che-3, osm-1, osm-6, tax-2*, *tax-4,* and *odr-4* mutants include the more limited sensory defects of *che-1* and *osm-8* mutants, which show reduced enhanced slowing. We hypothesize that the effects of specific sensory defects that cause reduced slowing are masked by broad defects that cause increased slowing.

Mutants with limited olfactory defects exhibit normal modulation of locomotion

Mutations in the guanyl cyclase *odr-1* and the glycosylated phosphatidylinositol linked signaling protein *odr-2* result in reduced detection of volatile odorants associated with AWC function (L'ETOILE and BARGMANN 2000; CHOU *et al.* 2001). *odr-1* mutants have a defect in the detection of isoamyl alcohol, benzaldehyde, and butanone, while *odr-2* mutants are defective in the detection of isoamyl alcohol and benzaldehyde (BARGMANN *et al.* 1993; L'ETOILE and BARGMANN 2000; CHOU *et al.* 2001). Both *odr-1(n1930)* and *odr-2(n1939)* mutants have normal enhanced slowing and thus defects in the detection of AWC-associated odorants alone do not appear to lead to modulation of locomotion defects (Figure 2.4).

Mutations of the capsaicin receptor homolog *osm-9*, the nuclear receptor *odr-7*, and the seven transmembrane receptor *odr-10* cause defects in the detection of volatile odorants associated with AWA function (SENGUPTA *et al.* 1994; SENGUPTA *et al.* 1996; COLBERT *et al.* 1997). *osm-9* and *odr-7* mutants have a defect in the detection of pyrazine and diacetyl (SENGUPTA *et al.* 1994; COLBERT *et al.* 1997), while *odr-10* mutants are defective in the detection of diacetyl (SENGUPTA *et al.* 1996). *osm-9* mutants are additionally defective in osmotic avoidance of sodium chloride (COLBERT and BARGMANN 1995; COLBERT *et al.* 1997; TOBIN *et al.* 2002). *osm-9(n1603)*, *odr-7(ky4)*, and *odr-10(ky32)* mutants exhibit normal enhanced slowing, suggesting that a defect in the detection of AWA-associated odorants alone does not lead to a modulation of locomotion defect (Figure 2.4).

Mutations in the G protein *odr-3* cause defects in the detection of AWA associated odorants but only some AWC detected odorants. *odr-3* mutants are defective in detecting diacetyl and pyrazine, detected by the AWA neurons, and benzaldehyde and

butanone, detected by the AWC neurons but still detect isoamyl alcohol, detected by the AWC neuron, and trimethylthiazole, which is detected by both of these neurons (BARGMANN *et al.* 1993; ROAYAIE *et al.* 1998). *odr-3(n2150)* mutants exhibit normal modulation of locomotion (Figure 2.4). These data suggest that animals that retain the ability to detect either AWA or AWC associated odorants do not exhibit increased slowing on a bacterial food source.

Multiple sensory processes detect locomotion-stimulating bacterial cues redundantly

Our data suggest that only gross defects in olfaction correlate with greater enhanced slowing and that more restricted olfactory defects do not lead to a modulation of locomotion defect (Table 2.1). We also find that mutations in genes that are expressed in the ASJ, ASK, ASG, and ASI neurons correlate with an inability to maintain locomotion on a bacterial food source (Table 2.2). Previous research shows that AWA and AWC neurons are involved in the detection of volatile odorants however the genes involved in maintaining *C. elegans* locomotion on bacteria are not uniformly expressed in the AWA and AWC neurons. We therefore hypothesize that either a single or multiple volatile components of bacteria are detected by the ASJ, ASK, ASG, and ASI neurons that act redundantly to maintain the locomotion of animals upon entering a bacterial lawn.

osm-3 and *osm-9* are also expressed in the ASJ, ASK, ASG, and ASI neurons, yet mutation of these genes has no effect on the enhanced slowing response (TABISH *et al.* 1995; COLBERT *et al.* 1997) (Table 2.2). It is possible that the specific function of these genes is not critical to this process. Laser ablation experiments could more rigorously define the roles of these sensory neurons in this process.

Mutations in the guanyl cyclase daf-11 suggest simple model for the role of chemosensation in dauer development

The decision to enter dauer is strongly influenced by the presence of dauer pheromone, which promotes dauer development, and bacteria, which inhibits dauer

development (GOLDEN and RIDDLE 1982; GOLDEN and RIDDLE 1984b). *daf-11* mutants have limited sensory defects and enter dauer development constitutively (VOWELS and THOMAS 1992). *daf-11*-associated sensory defects do not affect sensitivity to dauer pheromone (THOMAS *et al.* 1993). *che-3*, *osm-1*, and *osm-6* have more extensive sensory defects compared to *daf-11* mutants, yet do not enter dauer development constitutively (VOWELS and THOMAS 1992). In fact, these mutants fail to enter dauer under conditions that normally stimulate dauer development in wild-type animals (VOWELS and THOMAS 1992).

Since the formation of dauers is stimulated by dauer pheromone and lack of food, the simplest explanation for these findings is that *daf-11* mutants fail to detect food and *che-3*, *osm-1*, and *osm-6* mutants fail to detect dauer pheromone. In support of the second part of this hypothesis, multiple *che-3* mutant strains were also found to be resistant to the dauer inducing effects of dauer pheromone (JEONG *et al.* 2005). Since the sensory defects of *che-3*, *osm-1*, and *osm-6* mutants include those of *daf-11*, we hypothesize that *che-3*, *osm-1*, and *osm-6* mutants fail to detect both food and dauer pheromone. Since it appears that mutants that do not detect both food and pheromone fail to enter dauer, the inability to detect food signals might be masked by the inability to detect dauer pheromone with respect to dauer formation.

Dauer pheromone appears to be necessary and sufficient for dauer formation. Exogenous purified and synthetic dauer pheromone can stimulate dauer formation in wild-type animals in the presence of bacteria and is thought to inhibit a TGFβ signaling pathway, which in turn inhibits dauer formation (GEORGI *et al.* 1990; REN *et al.* 1996; RIDDLE and ALBERT 1997; JEONG *et al.* 2005). Mutations in the TGFβ signaling pathway block this signaling pathway, effectively achieving the same result as constitutive pheromone perception. It is not surprising then that the formation of constitutive dauers is not affected by additional mutations that cause sensory defects.

The limited sensory defects associated with *daf-11* mutants might result in an inability to detect food signals that inhibit dauer formation but leave the sensory pathways involved in the detection of dauer pheromone intact. Mutations in *che-3*, *osm-1*, and *osm-6* can suppress the dauer constitutive phenotype of *daf-11* as well as ablation of the AFD, ASE, ASH, ASJ, ASK, and ADL neurons, which suggest that these

neurons might be involved in the reception of dauer inducing signals (VOWELS and THOMAS 1992; MORI and OHSHIMA 1995; SCHACKWITZ *et al.* 1996). In comparison to other mutants with broad sensory defects, *daf-11* retains the ability to respond to many volatile odorants associated with AWB function. We speculate that AWB neurons might be involved in the detection of the volatile dauer pheromone. Two tests of this hypothesis are to determine whether the ablation of AWB neurons might also suppress the dauer constitutive phenotype of *daf-11* mutants and if restoration of AWB function in mutants with broad sensory defects restores sensitivity to dauer pheromone.

Volatile and soluble bacterial cues in context of foraging for food

Adult *C. elegans* likely detect many properties of bacteria. Animals that cannot detect any of the soluble or volatile chemicals mentioned can still detect some aspect of a bacterial lawn, because their locomotion rate is changed upon encountering it. Previous research has suggested that mechanosensory detection of a bacterial lawn might slow animals (SAWIN 1996).

Our hypothesis is that a bacterial lawn provides both locomotion maintaining and reducing cues for *C. elegans*. Detection of volatile bacterial chemicals might serve to maintain the locomotion of *C. elegans*, while detection of soluble bacterial chemicals might inhibit the locomotion of *C. elegans*. Early detection of a food source would likely involve volatile cues that can diffuse more easily through the environment, while soluble cues would be present only upon contact with that food source. In this context it would seem appropriate that volatile cues are required to sustain the locomotion of the animal approaching a food source, while soluble and mechanical cues would result in inhibition of locomotion since they are present only when the animal has entered a food source.

Future Directions

Since bacteria likely contain many cues that affect the locomotion of animals, a number of experiments might identify the chemical cues that affect *C. elegans* locomotion. First, to test whether secreted factors influence *C. elegans* locomotion, we

propose growing bacteria in a liquid culture and centrifuging the culture to remove all bacteria from the solution. We would then assay the locomotion of animals on plates that have been treated with bacteria-free conditioned media and unconditioned media. Second, we propose bacterial fractionation experiments to identify specific components of a bacterial food source that stimulate and suppress *C. elegans* locomotion. A third and complementary approach might include a bacterial genetic screen. We would mutagenize HB101 or OP50 *E. coli* strains, grow up mutagenized bacterial clones, look for altered *C. elegans* locomotory responses to these clones, and identify the bacterial genes change the rate of *C. elegans* locomotion when mutated.

Methods

Cultivation and Strains

All *Caenorhabditis elegans* strains were cultivated on NGM agar at 20°C as described by Brenner (BRENNER 1974), except that worms were grown on the *E. coli* strain HB101 as a food source. Worms were grown in non-crowded conditions. The wild-type *C. elegans* strain used was N2. Other strains used include PR674 *che-1(p674)*, CB1124 *che-3(e1124)*, CB3332 *che-12(e1812)*, MT4583 *odr-1(n1930)*, MT4592 *odr-2(n1939)*, MT5306 *odr-3(n2150)*, MT5300 *odr-4(n2144)*, CX4 *odr-7(ky4)*, CX32 *odr-10(ky32)*, PR808 *osm-1(p808)*, MT3600 *osm-3(p802)*, MT4304 *daf-11(m47ts)*, PR811 *osm-6(p811)*, MT3571 *osm-8(n1518)*, MT3602 *osm-9(n1603)*, PR691 *tax-2(p691)*, PR678 *tax-4(p678)*, PR767 *ttx-1(p767)*.

Locomotion Assay

Assays were conducted according to the protocol outlined by Sawin et al. (2000) with the exception that assay plates were made by spreading a drop of saturated HB101 culture using the smooth bottom of a small glass culture tube into the shape of a ring to minimize plate scarring. Assay plates were allowed to dry and were placed, along with

food-deprivation plates with no bacteria, at 37°C overnight. Only assay plates with minimal surface imperfections and a textured non-glassy appearing bacterial lawn were used. Animals were picked as L4 larvae and placed at 20°C 18-22 hours prior to the start of the assay. Assay plates and animals were allowed to equilibrate to room temperature before beginning the assay.

To begin the assay, ~10 animals were picked into a 24 well plate containing 1 ml of S basal per well. Using a 25 μL microcapillary tube and a mouth pipette, animals were washed free of bacteria and transferred to a second well. Animals were then immediately transferred to food-deprivation plates in a drop of liquid and freed from the droplet using a kimwipe. The off-food locomotion of animals was assayed on food-deprivation plates 5 minutes after being freed. Food-deprived animals were then picked off of food-deprivation plates using a microcapillary tube containing S basal and transferred to assay plates containing bacteria. Animals were assayed 5 minutes after being freed.

The locomotion of each animal was scored by counting the number of body bends made in a 20 second period of time. One body bend is the completion of one half of a sinusoidal wave propagated in either an anterior or posterior direction. Approximately 5 animals were scored on each plate.

Data Analysis

The average speed of a population was the mean speed of all animals tested. T values were determined using a student's t-test for comparing the means of two samples of unequal size. P-values were determined from a two-tailed student's t-distribution.

To graph the average locomotion rates for mutants assayed on different days in the same figure, we calibrated our results using paired wild-type controls collected on every assay day. First wild-type control data from all days were pooled to create a master wild-type dataset. A paired wild-type dataset was then created for each mutant by pooling the wild-type control data for each day that a specific mutant was assayed. To standardize data to the master control, we then multiplied the average speed of each mutant in both conditions (off-food, food-deprived) by the ratio between the master wild-type control and paired wild-type control (value Master / value Paired) for each

condition. Since we are only comparing each mutant to wild type, this method perfectly maintains the relationship of mutant to daily wild-type control while allowing us to show multiple results in one figure.

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Tables and Figures

Figure 2.1

che-1(p674) and *osm-8(n1518)* mutants slow less than wild type on bacteria after food-deprivation. Upon entering a bacterial food source in the food-deprived state wild-type animals exhibit an enhanced slowing response. *che-1(p674)* and *osm-8(n1518)* mutants move significantly faster than wild-type animals in the enhanced slowing condition. Error bars represent SEM. Asterisks indicate conditions in which the locomotion of mutants is significantly different from the wild type ($p < .001$).

Figure 2.1

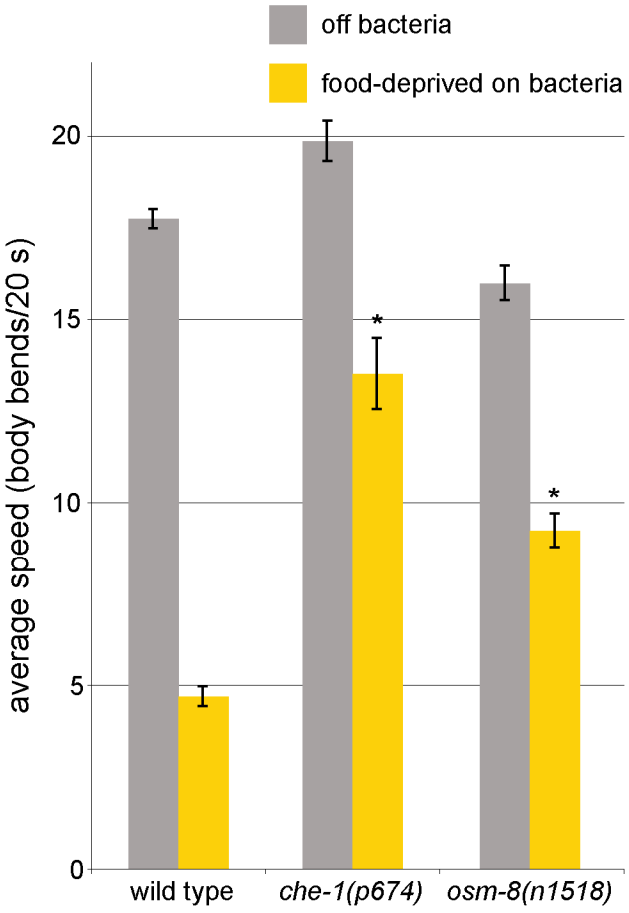
off bacteria
food-deprived on bacteria
average speed (body bends/20 s)
20
15
10
5
0
*
*
wild type
che-1(p674)
osm-8(n1518)

Figure 2.2

che-12(e1812) and *osm-3(p802)* mutants exhibit normal enhanced slowing. Upon entering a bacterial food source in the food-deprived state wild-type animals exhibit an enhanced slowing response. Like wild-type animals, *che-12(e1812)* and *osm-3(p802)* mutants exhibit normal enhanced slowing. Error bars represent SEM.

Figure 2.2

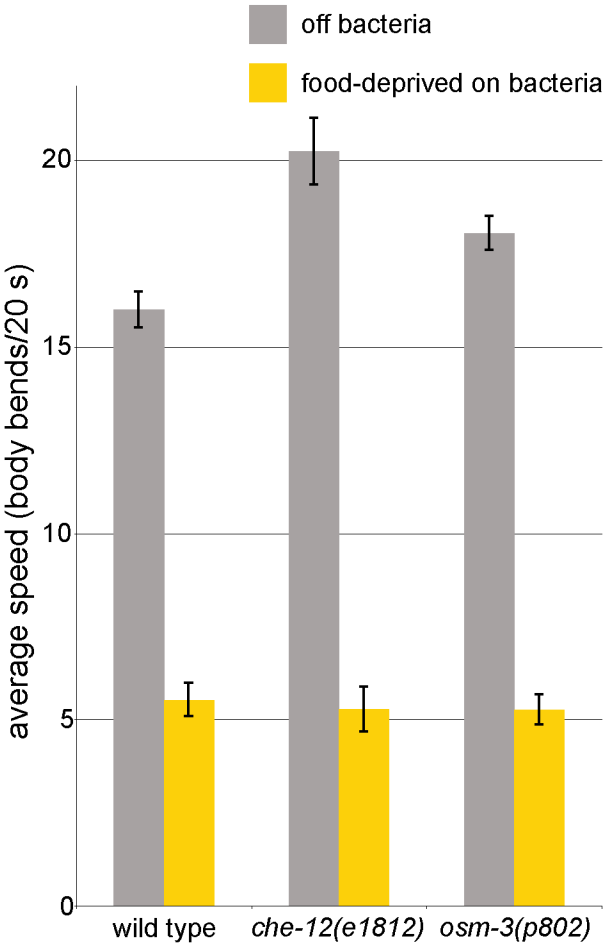
off bacteria
food-deprived on bacteria
average speed (body bends/20 s)
0
5
10
15
20
wild type
che-12(e1812)
osm-3(p802)

Figure 2.3

Mutants with broad defects in olfaction slow more than wild type on bacteria after food-deprivation. Upon entering a bacterial food source in the food deprived state wild-type animals exhibit an enhanced slowing response. *che-3(e1124)*, *odr-4(n2144)*, *osm-1(p808)*, *osm-6(p811)*, *tax-2(p691)*, and *tax-4(p678)* mutants move significantly slower than wild-type animals in the enhanced slowing condition. Error bars represent SEM. Asterisks indicate conditions in which the locomotion of mutants is significantly different from the wild type. ($p <$ *.005, **.001)

Figure 2.3

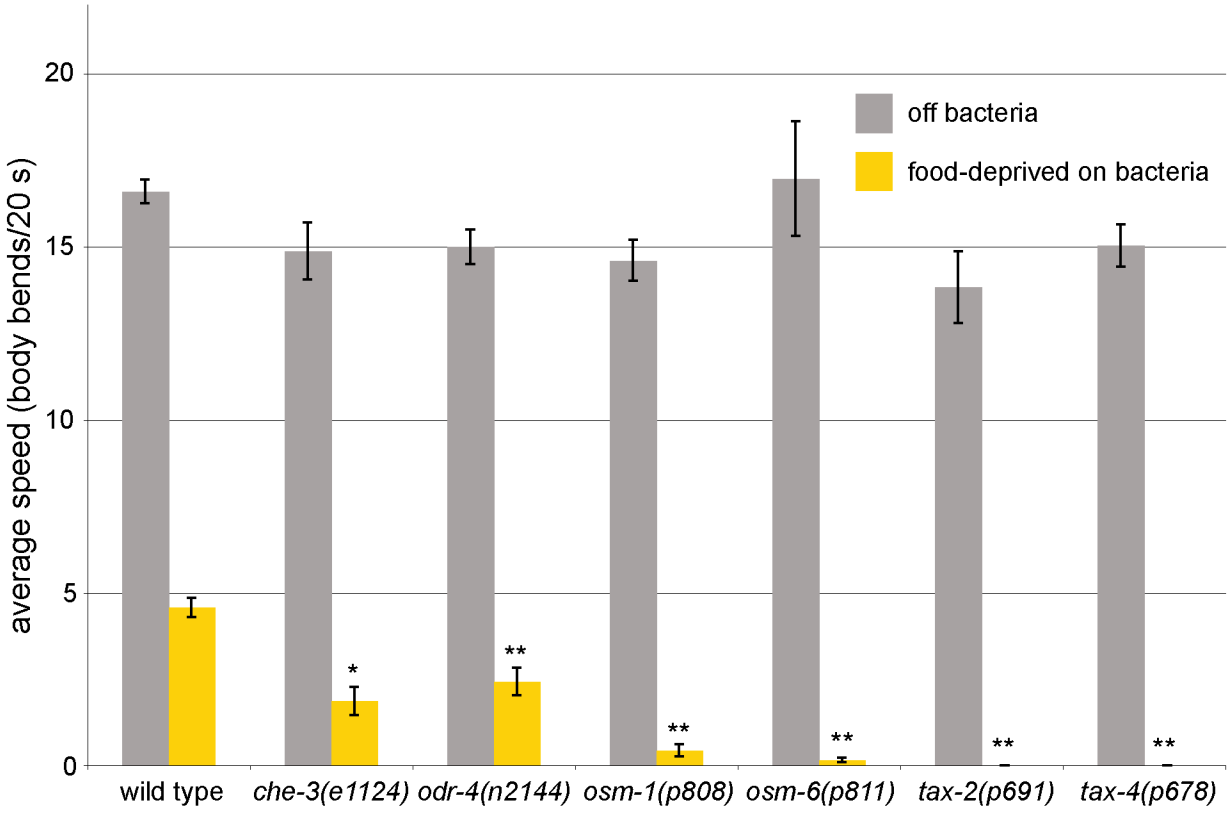
average speed (body bends/20 s)
20
15
10
5
0
off bacteria
food-deprived on bacteria
*
**
**
**
**
**
wild type
che-3(e1124)
odr-4(n2144)
osm-1(p808)
osm-6(p811)
tax-2(p691)
tax-4(p678)

Figure 2.4

Mutants with intermediate defects in the detection of volatile and soluble chemicals exhibit normal enhanced slowing. Upon entering a bacterial food source in the food deprived state wild-type animals exhibit an enhanced slowing response. *odr-1(n1930)*, *odr-2(n1939)*, *odr-3(n2150)*, *odr-7(ky4)*, *odr-10(ky32)*, and *osm-9(n1603)* mutants have various defects in the detection of volatile and soluble chemicals exhibit normal enhanced slowing. Error bars represent SEM.

Figure 2.4

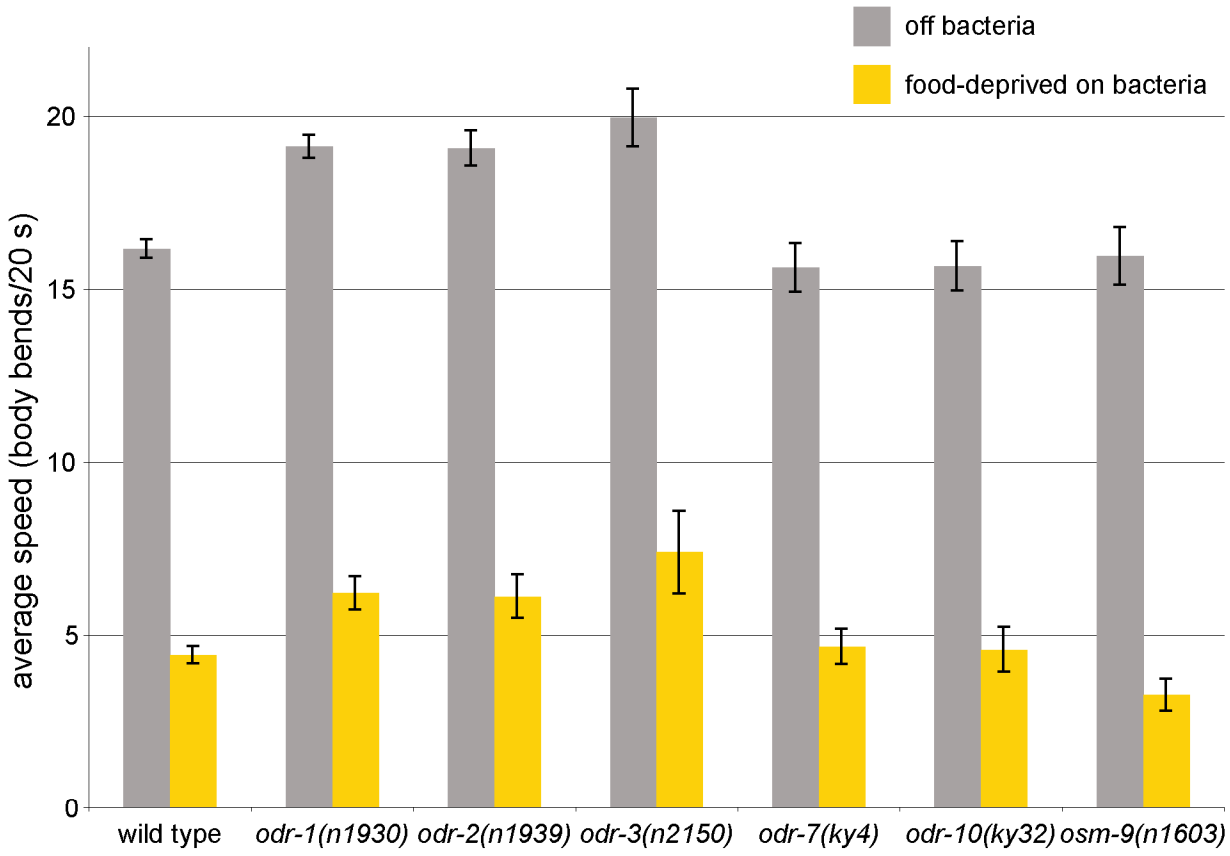
off bacteria
food-deprived on bacteria
average speed (body bends/20 s)
0
5
10
15
20
wild type
odr-1(n1930)
odr-2(n1939)
odr-3(n2150)
odr-7(ky4)
odr-10(ky32)
osm-9(n1603)

Table 2.1

Summary of sensory mutant locomotion and chemotaxis data. Table assembled from chemosensory data collected from primary research done by the community of *C. elegans* researchers. Citations indicate the primary research from which chemosensory data for each mutant was gathered. Chemosensory defects are highlighted in gray.

Table 2.1

			Condition										
Mutant	Citation	Locomotion	Osm (NaCl)	NaCl	Isoamyl Alcohol	Pyrazine	Diacetyl	TMT	Benzaldehyde	Butanone	Thermotaxis	Mec	Nose Touch
osm-1	1,5,7,9	reduced	defective	defective	defective	defective	defective	defective	defective	defective	normal	normal	defective
osm-6	1,5,7,9	reduced	defective	defective	defective	defective	defective	defective	defective	defective	normal	normal	defective
tax-2	1,2	reduced		defective	defective	defective	defective	defective	defective	defective			
tax-4	1,8	reduced		defective	defective	norm/defect	norm/defect	defective	defective	defective	defective		
che-3	1,5,7,9	slight reduc.	defective	defective	defective	defective	defective	defective	defective	defective	normal	normal	defective
odr-4	1,6	slight reduc.			normal	normal	defective	defective	defective	normal			
odr-2	1	normal	normal	normal	defective	normal	normal	normal	defective	normal			
che-12	1,7,9	normal	defective	normal	normal	normal	normal	normal	normal	normal	normal	normal	defective
odr-1	1	normal			defective	normal	normal	normal	defective	defective			
odr-10	12	normal			normal	normal	defective	normal	normal	normal			
odr-3	1,10	normal			normal/minor	minor/defect	defective	minor	defective	minor/defect			
odr-7	13	normal			normal	defective	defective	minor	normal	normal			
osm-3	1,5,7,9	normal	defective	minor	normal	normal	normal	normal	normal	normal	normal	normal	defective
osm-9	3,4,15	normal	defective		normal	defective	defective	normal	normal	normal			
ttx-1	11	normal									defective		
che-1/tax-5	1,16	increased	defective	defective	normal	normal	normal	normal	normal	normal			
osm-8	14	increased	defective										

Result Key

defective = strong reduction in behavioral index

highlights indicates strong defect

minor = small reduction in behavioral index

/ indicates instances where results from various studies are not in complete agreement

normal = no reduction in behavioral index

Citation Key

1 Bargmann CI, Hartweig EA and Horvitz HR (1993) Odorant-selective genes and neurons mediate olfaction in *C elegans* . Cell 74;515-527

2 Coburn CM, et al. (1998), A cyclic nucleotide-gated channel inhibits sensory axon outgrowth in larval and adult *C. elegans*: a distinct pathways for maintenance of sensory ... Devel. 125, 249-258

3 Colbert HA et al.: (1997). OSM-9, a novel protein with structural similarity to channels, is required for olfaction, mechanosensation, and olfactory adaptation in *C. elegans*. J. Neuroscience 17: 8259-8269

4 Colbert, HA, Bargmann, CI. (1995) Odornat-Specific Adaptation Pathways Generate Olfactory Plasticity in C. elegans. Neuron 14, 803-812.

5 Culotti JG and Russell RL (1978), Osmotic Avoidance Defective Mutants of the Nematode *Caenorhabditis elegans*, Genetics, 90(2):243

6 Dwyer ND, Troemel ER, Sengupta P, Bargmann CI. (1998) Odorant receptor localization to olfactory cilia is mediated by ODR-4, a novel membrane-associated protein. Cell. 1998 May 1;93(3):455-66.

7 Kaplan JM, Horvitz HR, A dual mechanosensory and chemosensory neuron in *Caenorhabditis elegans*, Proc Natl Acad Sci U S A. (1993) Mar 15;90(6):2227-31

8 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.

9 Perkins LA, Hedgecock EM, Thomson JN and Culotti JG (1986), Mutant sensory cilia in the nematode *C elegans* . Developmental Biology 117;456-487

10 Roayaie K. et al. (1998) The G alpha protein ODR-3 mediates olfactory and nociceptive function and controls cilium morphogenesis in *C. elegans* olfactory neurons. Neuron. Jan;20(1):55-67.

11 Satterlee JS et al.: (2001). Specification of the thermosensory neuron fate in *C. elegans* requires *ttx-1*, a homolog of otd/Otx. Neuron 31: 943-956.

12 Sengupta P, Chou JH, Bargmann CI. (1996). *odr-10* encodes a seven transmembrane domain olfactory receptor required for responses to the odorant diacetyl. Cell 84:899-909.

13 Sengupta P, Colbert HA, Bargmann CI. (1994). The *C. elegans* gene *odr-7* encodes an olfactory-specific member of the nuclear receptor superfamily. Cell 79:971-980.

14 Thomas JH Personal Communication

15 Tobin D, et al. (2002) Combinatorial expression of TRPV channel proteins defines their sensory functions and subcellular localization in *C. elegans* neurons. Neuron. Jul 18;35(2):307-18.

16 Uchida O, et al. The C. elegans *che-1* gene encodes a zinc finger transcription factor required for specification of the ASE chemosensory neurons. Devel. (2003) Apr;130(7):1215-24.

Table 2.2

Summary of sensory mutant locomotion and gene expression data. Table assembled from expression data that was collected by the community of *C. elegans* researchers. Citations indicate the primary research from which expression data for each mutant was gathered. Consistent expression is highlighted in green, variable or weak expression is highlighted in yellow, and the noted absence of expression is highlighted in white. The 'data type' category indicates the type of experiment upon which the expression data was based.

Table 2.2

			Neuron																		
Gene	Citation	Locomotion	ASE	ADL	ADF	ASH	ASI	ASJ	AFD	PHA	PHB	AQR	PQR	ASK	AWA	AWB	AWC	ASG	IL2	CEP	ADE	FLP
osm-1	9	reduced	strong	strong	strong	strong	strong	strong		strong	strong			strong				strong				
osm-6	4	reduced	strong	strong	strong	strong	strong	strong				strong	strong	strong				strong		strong	strong	
tax-2	2	reduced	strong				strong	strong	strong			strong	strong	strong		strong	strong	strong				
tax-4	6	reduced	strong				strong	strong	strong					strong			strong	strong				
che-3	17	slight reduc.	weak	strong	weak	strong	strong	strong	weak					strong	absent	absent	absent	weak			weak	weak
odr-4	5	slight reduc.		strong	strong	strong	strong	strong		strong	strong			strong	strong	strong	strong	strong				
odr-2	1	normal																strong	strong			
che-12		normal																				
odr-1	8,18	normal					strong	strong						strong		strong	strong					
odr-10	13	normal													strong							
odr-3	7,10	normal			variable	variable				strong	strong				variable	variable	variable					
odr-7	12	normal													strong							
osm-3	14	normal	strong	strong	strong	strong	strong	strong		strong	strong			strong				strong	strong			
osm-9	3	normal	strong	strong	strong	strong	strong	strong						strong	strong		strong	strong	strong			
ttx-1	11	normal							strong													
che-1/tax-5	16	increased	strong			strong																
osm-8	15	increased				strong																

Results Key Color Code — Strong Expression Reported — Weak/Variable Expression Reported — Expression Not Reported — Absense of Expression Reported

Citation Key	Data Type	
1	GFP	Chou JH et al.: 2001. The *Caenorhabditis elegans odr-2* gene encodes a novel Ly-6-related protein required for olfaction. Genetics 157: 211-224.
2	GFP	Coburn CM and Bargmann CI: 1996 . A putative cyclic nucleotide-gated channel is required for sensory development and function in *C. elegans*. Neuron 17 : 695-706.
3	GFP rescued	Colbert HA et al.: 1997. OSM-9, a novel protein with structural similarity to channels, is required for olfaction, mechanosensation, and olfactory adaptation in *C. elegans*. J. Neuro. 17: 8259-69
4	GFP	Collet J et al.: 1998. Analysis of *osm-6*, a gene that affects sensory cilium structure and sensory neuron function in *Caenorhabditis elegans*. Genetics 148: 187-200.
5	GFP Rescuec	Dwyer ND, et al. 1998 Odorant receptor localization to olfactory cilia is mediated by ODR-4, a novel membrane-associated protein. Cell. 1998 May 1;93(3):455-66.
6	GFP	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.
7	Antibody	Lans H et al.: 2004 . A Network of Stimulatory and Inhibitory G{alpha}-Subunits Regulates Olfaction in *Caenorhabditis elegans*. Genetics 167 : 1677-1687.
8	GFP/Ab	L'Etoile ND and Bargmann CI: 1997 . ODR-1 ENCODES A RECEPTOR GUANYLYL CYCLASE International*C. elegans* Meeting
9	GFP	McKay SJ et al.: 2004. Gene expression profiling of cells, tissues and developmental stages of the nematode *C. elegans*. Cold Spring Harbor Symposium on Quantitative Biology 68: 159-169.
10	GFP	Roayaie K et al.: 1998. The G-alpha protein ODR-3 mediates olfactoryand nociceptive function and controls cilium morphogenesis in *C. elegans* olfactory neurons. Neuron 20: 55-67.
11	GFP	Satterlee JS et al.: 2001. Specification of the thermosensory neuron fate in *C. elegans* requires *ttx-1*, a homolog of otd/Otx. Neuron 31: 943-956.
12	GFP	Sengupta P et al.: 1994. The C. elegans gene *odr-7* encodes an olfactory-specific member of the nuclear receptor superfamily. Cell 79: 971-980.
13	GFP	Sengupta P et al.: 1996. *odr-10* encodes a seven transmembrane domain olfactory receptor required for responses to the odorant diacetyl. Cell 84: 899-909.
14	lacZ	Tabish M et al.: 1995. Exclusive expression of *C. elegans osm-3* kinesin gene in chemosensory neurons open to the external environment. Journal of Molecular Biology 247: 377-389.
15		Thomas JH, Personal Communication
16	GFP	Uchida O et al.: 2003. The C. elegans *che-1* gene encodes a zinc finger transcription factor required for specification of the ASE chemosensory neurons. Development 130: 1215-1224.
17	GFP	Wicks SR et al.: 2000. CHE-3, a cytosolic dynein heavy chain, is required for sensory cilia structure and function in *Caenorhabditis elegans*. Developmental Biology 221: 295-307.
18	GFP	Yu S et al.: 1997. Guanylyl cyclase expression in specific sensory neurons: A new family of chemosensory receptors. Proceedings of the National Academy of Sciences USA 94: 3384-3387.

CHAPTER 3

The locomotion stimulating and suppressing effects of biogenic amines in *C. elegans* are balanced by reciprocal regulation

Summary

In the Mod Assay well-fed animals exhibit a minor basal slowing response upon encountering a bacterial food source. Food-deprived animals exhibit a greater enhanced slowing response upon encountering the same bacterial food source. Early experiments suggested that the basal slowing response required dopamine while the enhanced slowing response partially relied on serotonin to slow the animal. Using an automated locomotion tracking system that greatly improves the resolution at which we can study *C. elegans* locomotion, we re-investigated the roles dopamine and serotonin in these behaviors using mutants that were previously analyzed and a new mutant that specifically lacks serotonin. We also studied the role of the invertebrate norepinephrine analog octopamine and the related amine tyramine in these behaviors. Genetic analysis of mutations that eliminate the synthesis of serotonin, dopamine, octopamine, and tyramine suggest that an equilibrium between the locomotion stimulating and suppressing actions of these neurotransmitters is maintained by reciprocal regulation of each other.

Introduction

Beth Sawin's seminal graduate thesis entitled 'Genetic and Cellular Analysis of Modulated Behaviors in *Caenorhabditis elegans*' investigated the molecular and cellular mechanisms by which food deprivation affected the behavior of *C. elegans* (SAWIN 1996). In the Mod Assay, she found that well-fed and food-deprived animals moved at the same rate in the absence of bacteria. On a bacterial food source well-fed animals exhibit a 20% reduction in their locomotion rate while food-deprived animals exhibit a 70% reduction in their locomotion rate. The locomotion rates of well-fed and food-deprived animals on bacteria were named the basal and enhanced slowing responses, respectively (SAWIN *et al.* 2000).

A series of genetic, pharmacologic, and neuron ablation experiments showed that the basal slowing response relied on the neurotransmitter dopamine and the ADE, PDE, and CEP dopaminergic neurons (SAWIN *et al.* 2000). Serotonin was thought to function

exclusively in the enhanced slowing response. This second idea was supported by three key findings. The first finding was that mutants lacking serotonin and dopamine had strong defects in basal and enhanced slowing while mutants that lacked only dopamine had strong defects primarily in the basal slowing response (SAWIN *et al.* 2000). The second finding was that deletion of the serotonin reuptake transporter *mod-5* affected the enhanced slowing response and not the basal slowing response (RANGANATHAN *et al.* 2001). The third finding was that mutations in the serotonin gated chloride channel *mod-1* resulted in a specific reduction in the enhanced slowing response (RANGANATHAN *et al.* 2000). These data collectively suggested that dopamine and serotonin function in a non-overlapping manner with respect to basal and enhanced slowing.

Reduced serotonin signaling has been associated with a number of disorders in humans including hyper-aggressive states, stress disorders, substance abuse, depression, and others (COOPER *et al.* 2003). However in humans the majority of serotonin is present in the gut and appears to be related to digestive function and satiety signaling (DE PONTI 2004). In *C. elegans*, exogenous serotonin mimics the effects of a bacterial food source and induces egg laying, pharyngeal pumping, and reduced locomotion (HORVITZ *et al.* 1982). Thus serotonin appears to be involved in food-related responses in both worms and humans. Acting antagonistically to serotonin, the invertebrate noradrenergic analog octopamine inhibits egg-laying and pharyngeal pumping (HORVITZ *et al.* 1982). In insects, octopamine is thought to modulate a large number of physiological processes and functions as a stress hormone triggered by starvation and other environmental stresses (DAVENPORT 1984; ROEDER 1999).

Proper dopamine signaling has been shown to be important for a number of processes including coordinated movement and food-associated reward (JOG *et al.* 1999; SAPER *et al.* 2002). In *C. elegans*, dopamine signaling is involved in the detection of a bacterial lawn, as mentioned earlier, and in habituation to mechanical stimulus (SANYAL *et al.* 2004). After the administration of repeated taps to the side of a culture plate, animals initiate reversals less frequently in response to plate tap (RANKIN *et al.* 1990). Mutants with attenuated levels of dopamine exhibit less habituation than wild-type animals (SANYAL *et al.* 2004).

We investigated the roles of serotonin, dopamine, tyramine, and octopamine in the modulation of *C. elegans* locomotion using an automated tracking system that improves assay sensitivity and reproducibility. Our results extend our understanding of the roles of biogenic amines in the modulation of *C. elegans* locomotion and develop a new context within which to understand previous results.

Results

Dopamine-deficient cat-2 mutants exhibit reduced basal and enhanced slowing

cat-2 encodes a tyrosine hydroxylase that is required specifically for the synthesis of dopamine in *C. elegans* (Figure 3.1A). Mutations in *cat-2* result in animals with undetectable (SULSTON *et al.* 1975; LINTS and EMMONS 1999) or greatly reduced (SANYAL *et al.* 2004) levels of dopamine. Beth Sawin found that on a bacterial food source, *cat-2(e1112)* mutants moved significantly faster than wild-type animals in the well-fed state and slightly faster than wild type in the food-deprived state (SAWIN 1996). We found that the average locomotion rate of two independently isolated *cat-2* mutants, *cat-2 (e1112)* and *cat-2(n4547)*, exhibit significantly increased rates of locomotion on bacteria in both the well-fed and food-deprived states but not in the absence of food (Figure 3.1B). These findings demonstrate a role for dopamine in food-dependent slowing regardless of feeding state.

While we see statistically significant changes in the locomotion of some mutants in the absence of a bacterial food source, these changes tend to be smaller than in the on-bacteria conditions when we compare our mutants. We therefore focused our attention on the locomotion rates of animals on a bacterial food source and refer to locomotion in the absence of a bacterial food source only when interesting comparisons arise. In the case of *cat-2* mutants we note that dopamine deficiency causes these mutants to move faster on bacteria then in the absence of bacteria, a finding discussed in more detail in Chapter 4.

Serotonin deficient tph-1 mutants do not exhibit major defects in the modulation of locomotion

tph-1 encodes a tryptophan hydroxylase that is involved in the synthesis of serotonin (Figure 3.2A). Deletions of this locus result in mutants that lack serotonin but not dopamine (SZE *et al.* 2000). The canonical *tph-1(mg280)* mutant strain was never tested in the Mod assay because it exhibited uncoordinated locomotion (SZE *et al.* 2000). A second *tph-1(n4622)* allele, which contains a deletion of the *tph-1* locus, is not uncoordinated. Upon extensive backcrossing of the original *tph-1(mg280)* allele it was discovered that the uncoordinated behavior of this mutant was not due to mutation in the *tph-1* locus (Mark Alkema, personal communication). We therefore used the backcrossed *tph-1(mg280)* strain for all subsequent analysis.

The *tph-1(mg280)* and *tph-1(n4622)* mutants exhibited roughly normal modulation of locomotion, though *tph-1(mg280)* mutants might exhibit slightly reduced enhanced slowing consistent with the proposed role of serotonin in the enhanced slowing response (Figure 3.2B). The small but significant differences between the phenotypes of *tph-1(n4622)* and *tph-1(mg280)* mutants might reflect a difference in the genetic background of these two strains or the specific nature of each allele. The *mg280* mutation internally deletes half of the *tph-1* open reading frame (SZE *et al.* 2000), while *n4622* is a deletion of the transcriptional start site and approximately 90% of the open reading frame of *tph-1*. In subsequent analyses we used the *n4622* allele, as it is almost certainly a null allele. The finding that these mutants lack a large enhanced slowing defect was surprising, because serotonin signaling was thought to strongly contribute to the enhanced slowing response. This inconsistency led us to re-examine the older data regarding the roles of dopamine and serotonin in locomotory regulation in *C. elegans*.

Investigation of the role of serotonin in enhanced slowing

bas-1 cat-4 mutants likely retain some serotonin signaling and have defects in other signaling pathways

The role of serotonin in the modulation of *C. elegans* locomotion was previously assessed by comparing the dopamine deficient *cat-2* mutant and the *bas-1 cat-4* double mutant that was thought to only lack serotonin in addition to dopamine. *bas-1* encodes an aromatic amino acid hydroxylase that is involved in the synthesis of dopamine and serotonin (Figure 3.3A). *bas-1* mutants have reduced green formaldehyde-induced fluorescence (FIF) (LOER and KENYON 1993; SAWIN 1996), which indicates reduced levels of dopamine, and has reduced anti-serotonin immunoreactivity (WEINSHENKER *et al.* 1995). *cat-4* encodes a GTP cyclohydrolase I involved in the synthesis of a cofactor used by a large family of aromatic amino acid hydroxylases and nitric oxide synthases (WERNER-FELMAYER *et al.* 2002). This family of hydroxylases includes those that are involved in the synthesis of L-DOPA, 5-HTP, and octopamine (Figure 3.3A). L-DOPA and 5-HTP are the immediate precursors of dopamine and serotonin, respectively, and *cat-4* mutants have reduced levels of serotonin and dopamine (SULSTON *et al.* 1975; DESAI *et al.* 1988; WEINSHENKER *et al.* 1995). Since *bas-1* and *cat-4* mutants each have reduced levels of dopamine and serotonin, the double mutant was used because theoretically, levels of each neurotransmitter would be lower in the double mutant than in either of the single mutants.

As discussed earlier, mutations in the *C. elegans* tryptophan hydroxylase gene *tph-1* disrupt the synthesis of serotonin and not dopamine (SZE *et al.* 2000). We find that the *bas-1 cat-4* double mutants was different from that of the *tph-1 bas-1 cat-4* triple mutant in that the triple mutant had significantly increased locomotion in the off-bacterial condition and possibly reduced locomotion in the food-deprived condition on bacteria (Figure 3.3B). Furthermore, the *bas-1 cat-4* mutant does not exhibit the egg laying defects of the *tph-1* mutant (WEINSHENKER *et al.* 1995; SZE *et al.* 2000) while the *tph-1 bas-1 cat-4* triple mutant does (data not shown). Therefore it is likely that the *bas-1 cat-4* double mutant has residual levels of serotonin that are sufficient to modulate *C. elegans* locomotion. We also now suspect that in addition to serotonin synthesis, other signaling pathways might additionally be disturbed by the *cat-4* mutation.

Mutations in the serotonin reuptake transporter gene mod-5 result in serotonin dependent reduction of locomotion in the basal and enhanced slowing response

Although depletion of serotonin has very little effect on food-dependent locomotion, mutations in the serotonin reuptake transporter *mod-5* lead to increased food-dependent slowing (SAWIN 1996). In fact, *mod-5* mutants become paralyzed upon entering a bacterial lawn in the enhanced slowing response (SAWIN 1996; RANGANATHAN *et al.* 2001). Since exogenous serotonin stimulates food-related behaviors in *C. elegans*, including the suppression of locomotion (HORVITZ *et al.* 1982), it is believed that when the worm encounters a bacterial lawn that serotonin is released which slows the animal (Figure 3.4A). One model proposed that inactivation of the serotonin reuptake transporter *mod-5* results in reduced ability to clear serotonin from the synapses, thus potentiating the locomotion-inhibiting effects of serotonin (RANGANATHAN *et al.* 2001).

Initially it was reported that a newly isolated *mod-5(n3314)* deletion allele caused defects in only the enhanced slowing response, which supported the idea that serotonin had a specific role only in enhanced slowing (RANGANATHAN *et al.* 2001). Subsequent experiments have shown that *mod-5(n3314)* mutants, like all previously isolated *mod-5* mutants (SAWIN 1996; RANGANATHAN *et al.* 2001), exhibited increased slowing in both the basal and enhanced slowing condition (Figure 3.4B). Furthermore, the locomotion of *mod-5(n3314)* mutants in the absence of a bacterial food source was also significantly reduced.

The locomotion defects associated with *mod-5(n3314)* are completely suppressed by the addition of a mutation in *tph-1* (Figure 3.4B). This experiment supports the finding that *mod-5* is indeed a serotonin specific transporter (RANGANATHAN *et al.* 2001). Our findings are consistent with the model in which excess serotonin signaling in *mod-5* mutants leads to a greater suppression of locomotion because serotonin can not be effectively cleared from intracellular spaces.

Mutations in the serotonin-gated chloride channel gene mod-1 do not affect the modulation of C. elegans locomotion

To study how food deprivation modulates *C. elegans* locomotion, Beth Sawin performed a genetic screen for mutants that exhibited reduced enhanced slowing. In this

screen she isolated a number of mutants including a strain containing a mutation in the serotonin-gated chloride channel *mod-1(n3034)*. This original isolate exhibited reduced enhanced slowing and resistance to immobilization by exogenous serotonin (SAWIN 1996). By following the serotonin-resistance phenotype in a series of genetic mapping experiments, *mod-1* was determined to be a serotonin-gated chloride channel (RANGANATHAN *et al.* 2000).

Our automated tracking system confirmed that the original screen isolate containing the *mod-1(n3034)* mutation had reduced enhanced slowing; however, two mutants containing independently isolated deletion alleles of the serotonin gated chloride channel, *mod-1(ok103)* and *mod-1(nr2043)*, did not exhibit reduced enhanced slowing (Figure 3.5 A,B). Upon further investigation we found that the *mod-1(n3034)* point mutation and the enhanced slowing defect of the original isolate were genetically separable. We outcrossed the original *mod-1(n3034)* strain and isolated two strains. One strain contained the point mutation *n3034* in the serotonin gated chloride channel *mod-1* and exhibited normal modulation of locomotion and the second strain exhibited an enhanced slowing defect but did not contain the *n3034* point mutation (Figure 3.5 A,B). The mutation in the uncloned locus responsible reduced enhanced slowing now has the new allele designation *n4954*.

While all mutations in the serotonin-gated chloride channel gene *mod-1* cause strong resistance to immobilization by exogenous serotonin, no mutations in *mod-1* result in reduced enhanced slowing (Figure 3.5 A-D).

Tyramine alone or together with octopamine generally stimulates C. elegans locomotion

Tyrosine is decarboxylated by the *C. elegans* tyrosine decarboxylase *tdc-1* to make tyramine, which is then hydroxylated by a tyramine β-hydroxylase *tbh-1* to make octopamine (Figure 3.6A); *tdc-1* mutants lack both tyramine and octopamine, while *tbh-1* mutants lack only octopamine (ALKEMA *et al.* 2005). *tbh-1* mutants are also reported to have increased levels of tyramine (ALKEMA *et al.* 2005). While *tbh-1* mutants, which lack only octopamine, exhibit relatively normal modulation of locomotion, *tdc-1* mutants,

which lack both neurotransmitters have reduced locomotion rates under all conditions (Figure 3.6B). Thus, either tyramine alone or octopamine and tyramine acting redundantly appear to be involved in stimulating *C. elegans* locomotion generally on a bacterial food source.

Mutants lacking dopamine, serotonin, octopamine, and tyramine exhibit grossly normal modulation of locomotion

We found that the *cat-2 tph-1 tdc-1* triple mutant lacking dopamine, serotonin, octopamine, and tyramine exhibited relatively normal modulation of locomotion when compared to mutants lacking only one or two of these neurotransmitters either alone or in pairs (Figure 3.7). These data suggest that these signaling pathways exist in a balanced state and that perturbation of this equilibrium has a greater effect on activity than removing these biogenic amines altogether.

Additionally, the biogenic amines dopamine, serotonin, tyramine, and octopamine appear to be dispensable for the ability of animals to respond to a bacterial food source and food-deprivation. We hypothesize that biogenic amine signaling increases the adaptability of *C. elegans* by allowing a greater number of environmental factors to modulate its motor program.

A role for serotonin in the inhibition and stimulation of locomotion is uncovered in mutants lacking other biogenic amines

Pharmacological experiments have shown that exogenous serotonin suppresses the locomotion of animals (HORVITZ *et al.* 1982; RANGANATHAN *et al.* 2000). Increasing endogenous serotonin signals through mutation of the serotonin reuptake transporter gene *mod-5* also causes a reduced rate of locomotion that is dependent on *tph-1* (Figure 3.4B). Comparing the tyramine- and octopamine-deficient *tdc-1* single mutant to the *tph-1 tdc-1* double mutant, which also lacks serotonin, and the dopamine-, tyramine-, and octopamine-deficient *cat-2 tdc-1* double mutant, to the *cat-2 tph-1 tdc-1* triple mutant, which also lacks serotonin, we saw that removing serotonin with the addition of a

mutation in *tph-1* results in a general increase in locomotion (Figure 3.8A). These results further support a role for serotonin in inhibiting *C. elegans* locomotion.

Comparing the *cat-2* single mutant to the *cat-2 tph-1* double mutant, however, we saw that removing serotonin with the addition of a *tph-1* mutation in a *cat-2* mutant background has the effect of reducing locomotion in both the basal and enhanced slowing response (Figure 3.8B). Apart from the known role of serotonin in the inhibition of locomotion, these data suggest a role for serotonin in the stimulation of *C. elegans* locomotion.

Serotonin and octopamine/tyramine are mutually dependent on the function of each other to stimulate locomotion

As we reported earlier, *tdc-1* mutation results in loss of octopamine/tyramine signaling and generally represses locomotion indicating that the endogenous function of octopamine/tyramine is to stimulate the locomotion of animals. However, comparing the locomotion of the serotonin deficient *tph-1* single mutant to the *tph-1 tdc-1* double mutant, we find that the addition of a *tdc-1* mutation in a *tph-1* mutant background has no effect, or might even result in slightly elevated locomotion (Figure 3.9A). Thus, the locomotion stimulating activity of *tdc-1* is dependent on the presence of *tph-1* activity. These data are consistent with octopaminergic/tyraminergic signaling stimulating *C. elegans* locomotion by inhibiting or antagonizing serotonin signaling, which can repress locomotion (Figure 3.9B).

As we reported earlier, *tph-1* can also stimulate locomotion when dopamine is absent. Comparing the *cat-2* and *cat-2 tph-1* double mutant we saw that the addition of a *tph-1* mutation in a *cat-2* background reduced locomotion in both the basal and enhanced slowing responses. This is not the case when *tdc-1* is also mutated. Comparing *cat-2 tdc-1* to *cat-2 tph-1 tdc-1* triple mutant we find that addition of a *tph-1* mutation in a *cat-2 tdc-1* mutant background does not reduce locomotion but now slightly increases locomotion in both the basal and enhanced slowing response (Figure 3.9C). Thus it appears that the locomotion stimulating activity of *tph-1* is dependent on a functioning copy of *tdc-1*. These data are consistent with serotonin signaling stimulating locomotion

through the regulation of octopamine/tyramine signaling, which stimulates locomotion (Figure 3.9D). A model of this reciprocal regulation is proposed later.

The inhibition of locomotion by dopamine signaling is dependent on the presence of octopamine/tyramine

As we reported earlier, *cat-2* mutations result in the loss of dopamine and greatly increased locomotion in the basal and enhanced slowing conditions. Comparing the serotonin-deficient *tph-1* single mutant to the *cat-2 tph-1* double mutant and the octopamine/tyramine-deficient *tdc-1* single mutant to the *cat-2 tdc-1* double mutant, we see that addition of the *cat-2* mutation causes a significant increase in locomotion in a *tph-1* background but not in the *tdc-1* mutant background (Figure 3.10A). Thus *cat-2* requires the function of *tdc-1* to inhibit locomotion. These data are consistent with a model in which *cat-2* inhibits locomotion through the regulation of *tdc-1* (Figure 3.10B).

Discussion

Studying mutants that have defects in the synthesis of serotonin, dopamine, octopamine, and tyramine, we find a complex set of genetic interactions that reveal roles for each of these neurotransmitters in both basal and enhanced slowing responses. Surprisingly we find that mutants lacking all of these neurotransmitters exhibit relatively normal modulation of locomotion while perturbing the synthesis of a subset of biogenic amines dramatically changes behavior. Our results are consistent with a simple model in which the locomotion stimulating and inhibiting effects of octopamine/tyramine and serotonin are balanced by reciprocal regulation of each other. Dopamine appears to regulate octopamine/tyramine signaling.

Dopamine and serotonin are involved in both basal and enhanced slowing

Previously it had been reported that dopamine deficient *cat-2(e1112)* mutants had reduced basal slowing and normal (SAWIN *et al.* 2000) or slightly reduced (SAWIN 1996) enhanced slowing. Ablation of the ADE, PDE, and CEP dopaminergic neurons resulted in operated animals that had a similar defect in the basal slowing response (SAWIN *et al.* 2000). These animals were never tested in the enhanced slowing condition (SAWIN *et al.* 2000). Our automated tracking system shows that *cat-2(e1112)* animals and a newly isolated *cat-2(n4547)* deletion mutant move faster than wild-type animals in the basal slowing response and in the enhanced slowing response while their locomotion in the absence of bacteria is relatively unaffected. These data suggest that dopamine signaling is either directly or indirectly involved in both the basal and enhanced slowing responses.

In our experiments we re-examined the three key findings that supported a role for serotonin signaling in the enhanced slowing response specifically. First is the notion that removing serotonin affects only the enhanced slowing response. We found that *tph-1* mutants, which lack serotonin but have dopamine, exhibit normal modulation of locomotion. Early conclusions about the role of serotonin were based on comparisons between the dopamine-deficient *cat-2* mutant and the *bas-1 cat-4* double mutant, which was thought to lack only serotonin and dopamine. We now know that the *bas-1 cat-4* double mutant contains enough residual serotonin to affect the locomotion of the animal and that *cat-4* encodes a GTP cyclohydrolase I which is the rate limiting enzyme in the synthesis of tetrahydrobiopterin which is an essential cofactor required by nitric oxide synthases, phenylalanine hydroxylases, tyrosine hydroxylases, and tryptophan hydroxylase (WERNER-FELMAYER *et al.* 2002). This last contention brings into question the assumption that the difference in the behavior between *cat-2* and *bas-1 cat-4* mutants is specifically due to removal of serotonin.

Second, we find that like all previously tested *mod-5* reuptake transporter mutants, *mod-5(n3314)* mutants have reduced locomotion in both basal and enhanced slowing responses. This result is confirmed by manual and automated scoring of *mod-5(n3314)* locomotion in multiple assays and in the context of multiple genetic screens performed in a *mod-5(n3314)* background. Third, we found that all strains containing *mod-1* mutations except the original *n3034* isolate have normal modulation of locomotion. From the original *mod-1* isolate we were able to genetically separate the

n3034 mutation in the serotonin-gated chloride channel *mod-1* from mutation that causes the modulation of locomotion defect.

We believe that the early conclusions were complicated by assumptions that were made about the mutants studied and the difficult nature of quantifying small differences in locomotion rate by manual scoring methods. In light of these new findings we no longer believe that serotonin signaling acts to only suppress locomotion in the enhanced slowing response. We now believe that serotonin signaling can both suppress and stimulate *C. elegans* locomotion in both the basal and enhanced slowing response.

Genetic interaction data suggest a model for the regulation of C. elegans locomotion

We see that perturbing the synthesis of biogenic amines in *C. elegans* can have very different effects on the locomotion of *C. elegans* depending on whether the synthesis of each neurotransmitter is disrupted separately or in combinations. We look at how the locomotion of mutants change when each of these neurotransmitters are removed by genetic mutation and create a model of the genetic interactions between *cat-2*, *tph-1*, and *tdc-1* in the basal and enhanced slowing response separately. Looking at how those interactions change from basal to enhanced slowing conditions gives us insight into how the roles of these genes change with food deprivation.

In the basal slowing condition we found that *tdc-1* stimulated locomotion through the inhibition of *tph-1*. We also found that *tph-1* can suppress locomotion as well as stimulate locomotion in a *tdc-1* dependent fashion. These interactions can be represented by a genetic regulatory circuit in which *tph-1* and *tdc-1* mutually regulate each other (Figure 3.11A). We have also shown that *cat-2* inhibits locomotion through regulation of *tdc-1* (Figure 3.11A).

In the enhanced slowing response *cat-2* mutants have increased locomotion, *tph-1* mutants have no change in locomotion, and *tdc-1* mutants have decreased locomotion. With respect to the enhanced slowing response mutations in *tph-1* are epistatic to mutations in *tdc-1*, which are epistatic to mutations in *cat-2*. These genetic interactions form a linear pathway in which *cat-2* inhibits *tdc-1*, which inhibits *tph-1*, which inhibits locomotion (Figure 3.11B). The genetic interactions we see in the enhanced slowing

condition are also represented in the basal slowing model. Our enhanced slowing model differs in that the regulation of *tdc-1* by *tph-1*, and *tdc-1* independent stimulation of locomotion, are absent. As with all genetic pathway analyses, there is always the formal possibility that all of these genes exert their effects on locomotion in parallel pathways.

In *C. elegans* serotonin signaling has been associated with bacteria-induced behaviors (HORVITZ *et al.* 1982), and thus it has been proposed that serotonin is released in the presence of a bacterial lawn. We hypothesize that in the absence of food, serotonin is no longer released, which would cause the regulatory effects of serotonin signaling to diminish. In our genetic model of enhanced slowing, we saw that after animals are food-deprived, *tph-1* no longer regulates *tdc-1* which likely reflects reduced regulatory effects of serotonin signaling on octopamine/tyramine signaling after food-deprivation (Figure 3.11B).

In C. elegans biogenic amines might function like a simplified autonomic nervous system that modulates activity

The complementary actions of the sympathetic and parasympathetic nervous system generally prepare the body for action or for food consumption and reproduction, respectively (LOEWY and SPYER 1990). The sympathetic arm of the autonomic nervous system coordinates 'fight or flight' responses, while the parasympathetic arm of the autonomic nervous system coordinates 'rest and digest' responses (CANNON 1927). The sympathetic arm of the autonomic nervous system relies primarily on the peripheral action of norepinephrine, which prepares the body for action by increasing the rate and strength of heart contractions, increasing pulmonary function, dilating pupils, and reducing digestive functions (LOEWY and SPYER 1990). The parasympathetic arm of the autonomic nervous system acts on the same processes in an opposing fashion and slows heart rate, restores normal pulmonary function, restores normal pupil dilation, and increases digestive functions (LOEWY and SPYER 1990).

In *C. elegans*, serotonin is involved in feeding and reproductive behavior, while the invertebrate norepinephrine analog octopamine acts as a stress hormone in invertebrates (DAVENPORT 1984; ROEDER 1999) and has an opposing effect on these

behaviors (HORVITZ *et al.* 1982). Serotonin has been shown to be involved in the stimulation of egg-laying, stimulation pharyngeal pumping, and inhibition of locomotion (HORVITZ *et al.* 1982; TRENT *et al.* 1983; SZE *et al.* 2000; RANGANATHAN *et al.* 2001), while octopamine has been shown to be involved in the inhibition of egg-laying, pharyngeal pumping, and stimulation of locomotion (HORVITZ *et al.* 1982; ALKEMA *et al.* 2005). The complementary actions of octopamine and serotonin in coordinating food, reproductive, and stress-related behaviors have strong parallels to the functional and complementary actions of the sympathetic and parasympathetic nervous system in humans. Our studies provide genetic evidence that supports the idea that octopamine/tyramine signaling antagonizes serotonin signaling and that serotonin and octopamine/tyramine co-regulate the function of each other.

In Parkinson's patients, loss of dopamine signaling results in autonomic failure and symptoms that reflect both increased sympathetic and parasympathetic activity (AMINOFF and WILCOX 1971; POEWE 2006; DUBOW 2007). Thus, dopamine also appears to be involved in maintaining balance between sympathetic and parasympathetic drives in the autonomic nervous system. In *C. elegans*, dopamine also appears to result in misregulation of octopamine and serotonin signaling that results in highly abnormal activity levels.

Future Directions

Our results suggest that serotonin, dopamine, octopamine, and tyramine have distinct and separable roles in the different circuits in which they act. Mutations in putative metabotropic and ionotropic amine receptors are rapidly being isolated and characterized. Genetic studies of biogenic amine receptor function has the potential to separate the roles of each biogenic amine in the regulation of *C. elegans* locomotion. By determining which biogenic amines activate each of these receptors and then identifying the specific processes that these receptors are involved has the potential to parse the broad roles currently assigned to serotonin, dopamine, octopamine, and tyramine in locomotion.

Determining the expression patterns of these receptors and laser ablation of receptor-expressing cells will be the first steps in identifying the important cells and circuits that are involved in the regulation of *C. elegans* locomotion.

Methods

Cultivation and Strains

Caenorhabditis elegans strains were cultivated on NGM agar at 20°C as described by Brenner (1974), except that worms were grown on *E. coli* strain HB101 as a food source. The wild-type *C. elegans* strain used was N2. Strains used include CB1112 *cat-2(e1112)*, GR1321 *tph-1(mg280) non-backcrossed*, MT10661 *tdc-1(3420)*, MT11374 *tbh-1(n3722)*, MT14984 *tph-1(n4622)*, MT15434 *tph-1(mg280) backcrossed*, MT15612 *cat-2(n4547) tph-1(n4622)*, MT15619 *mod-5(n3314) tph-1(n4622)*, MT15620 *cat-2(n4547)*, MT16044 *cat-2(n4547) tdc-1(n3420)*, MT16045 *tph-1(n4622) tdc-1(n3420)*, MT16653 *n4954*, MT16873 *tph-1(n4622) bas-1(ad446) cat-4(e1141)*, MT16874 *cat-2(n4547) tph-1(n4622) tdc-1(n3420)*, MT8541 *mod-1(n3034);n4954*, MT8943 *bas-1(ad446) cat-4(e1141)*, MT9667 *mod-1(nr2043)*, MT9668 *mod-1(ok103)*, MT9772 *mod-5(n3314)*, MT18532 *mod-1(n3034)*.

Locomotion Assay

Assays were conducted according to Sawin et al. (2000) with the following modifications. Assay plates containing no bacteria or with a ring shaped bacterial lawn were examined, and only those with minimal surface imperfections and a textured non-glassy appearing bacterial lawn were used for locomotion assays. Assays were performed in a temperature-controlled room at 22°C. The locomotion of 20-25 animals was recorded in each assay. Recording of locomotion was scored by an automated locomotion tracking system and began 5 min after animals were transferred to assay plates and lasted for 2 min.

We used a Sony XCD X710 firewire camera at 1024x768 resolution to capture high contrast dark field images of our assay plates illuminated from the side by a ring of LEDs. Images were acquired at 1 Hz. The centers of mass (centroids) of all worms were found and individual position recordings were assembled into tracks from centroid positions using the methods outlined by Clark et al. (2007). Animal speeds were calculated by dividing the path distance an animal traveled by the time it took to travel that path. Each speed recording was calculated by averaging 5 speed measurements observed during a 5 sec sliding window to minimize the noise in centroid displacements. Such instantaneous speed measurements varied from 0 to roughly 0.3 mm per sec.

Data Analysis

The average speed of a population was calculated to be the mean of all instantaneous speeds calculated for all animals tested. T values were determined using a student's t-test for comparing the means of two samples of unequal size. P-values were determined from a two-tailed student's t-distribution.

To compare the average locomotion rates for mutants assayed on different days, we calibrated our results using paired wild-type controls collected on every assay day. First, wild-type control data from all days were pooled to create a master wild-type dataset. A paired wild-type dataset was then created for each mutant, by pooling the wild-type control data for each day that specific mutant was assayed. To standardize data to the master control, we then multiplied the average speed of each mutant in each condition (off-food, well-fed, food-deprived) by the ratio between the master wild-type control and paired wild-type control (value Master / value Paired) for each condition. We found that this method most successfully maintained the integrity of the relationships between mutants when compared to data collected completely in parallel.

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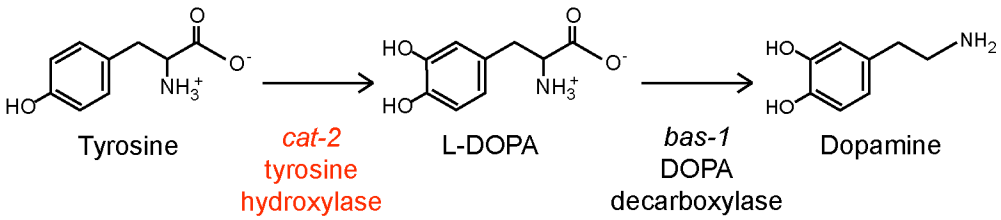
Figures

Figure 3.1

cat-2 mutants have defects in the synthesis of dopamine and increased locomotion on bacteria when compared to wild-type animals. (A) *cat-2* encodes the *C. elegans* tyrosine hydroxylase enzyme involved in the synthesis of dopamine. (B) Average velocities of wild-type, *cat-2(n4547)*, and *cat-2(e1112)* animals under each condition. Error bars represent SEM. Asterisks indicate instances where the locomotion of the mutant is significantly different from wild-type locomotion in the same condition ($p < .001$).

Figure 3.1

A.


Tyrosine
cat-2
tyrosine
hydroxylase
L-DOPA
bas-1
DOPA
decarboxylase
Dopamine

B.

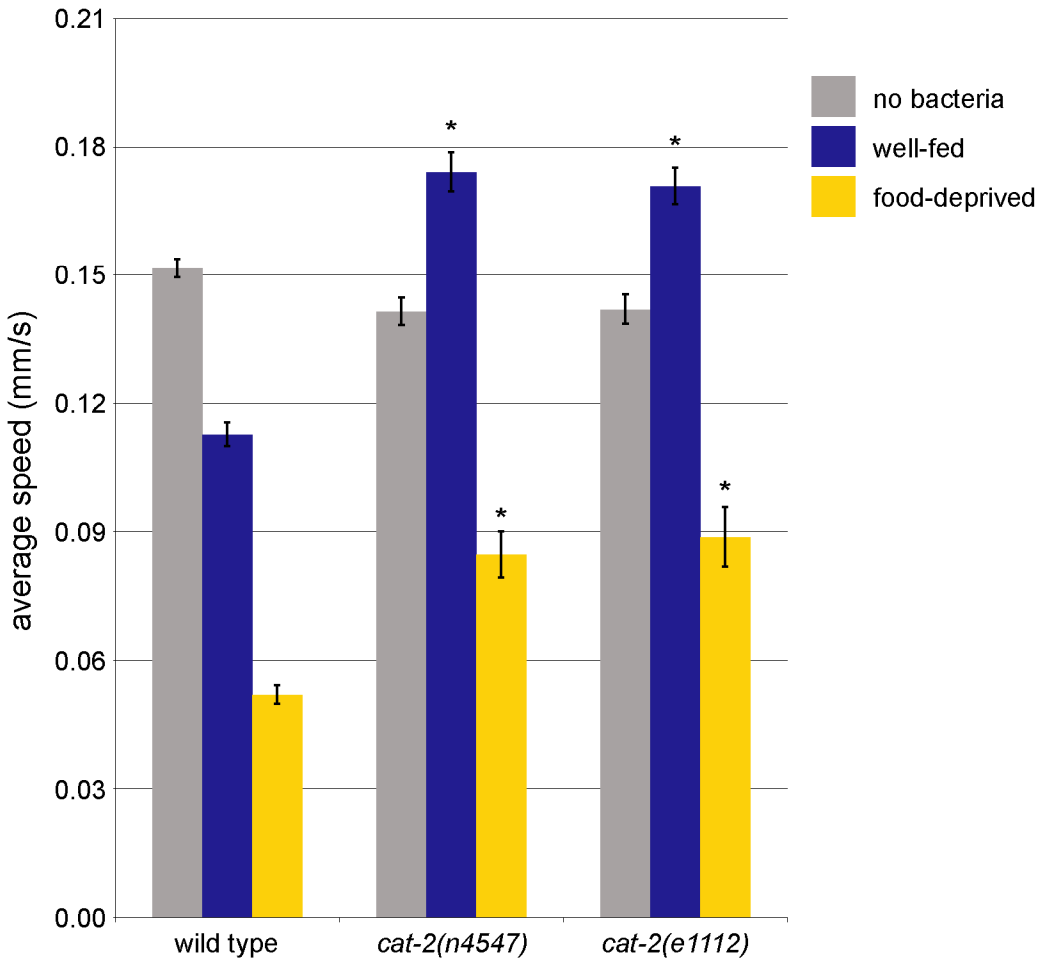
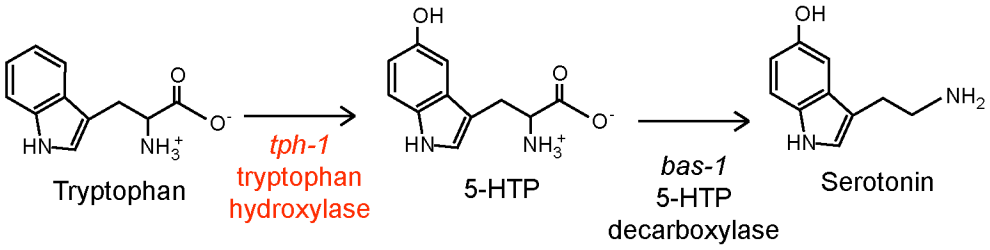

average speed (mm/s)
0.21
0.18
0.15
0.12
0.09
0.06
0.03
0.00
no bacteria
well-fed
food-deprived
wild type
cat-2(n4547)
cat-2(e1112)

Figure 3.2

tph-1 mutants have defects in the synthesis of serotonin and exhibit only slightly altered locomotion on a bacterial food source compared to wild-type locomotion. (A) *tph-1* encodes the *C. elegans* tryptophan hydroxylase required for the synthesis of serotonin. (B) Average velocities of wild-type, *tph-1(n4622)*, and *tph-1(mg280)* backcrossed animals under each condition. Error bars represent SEM. Asterisks indicate instances where the locomotion of the mutant is significantly different from wild-type locomotion in the same condition ($p < *.01$, **.001).

Figure 3.2

A.


OH
O
O⁻
HN
NH3+
NH2
Tryptophan
tph-1
tryptophan
hydroxylase
5-HTP
bas-1
5-HTP
decarboxylase
Serotonin

B.

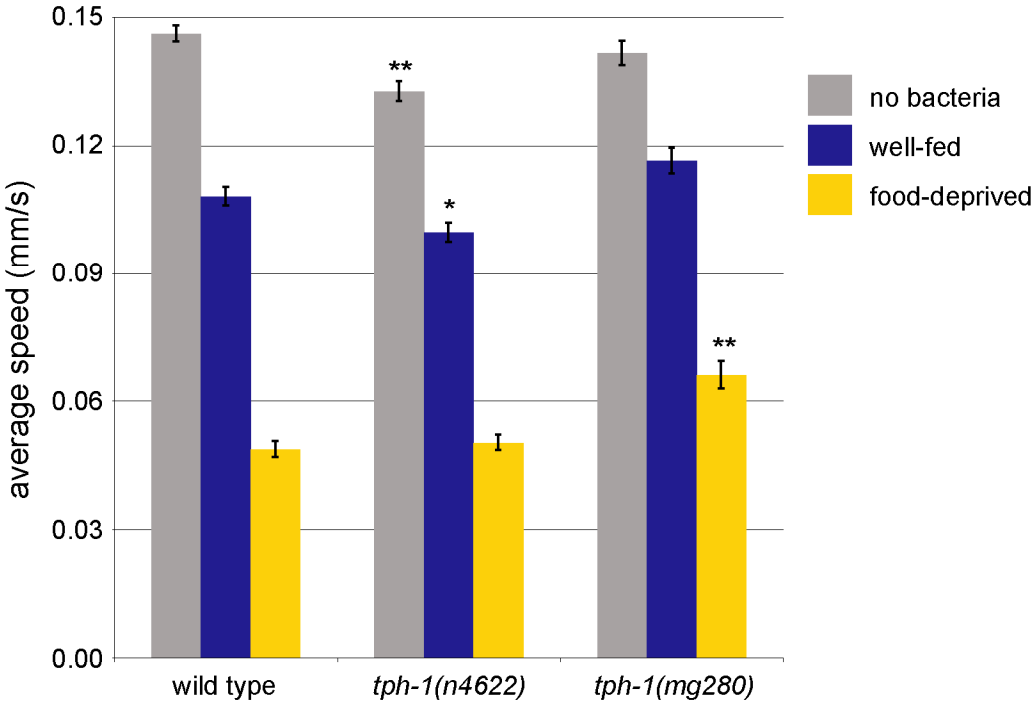
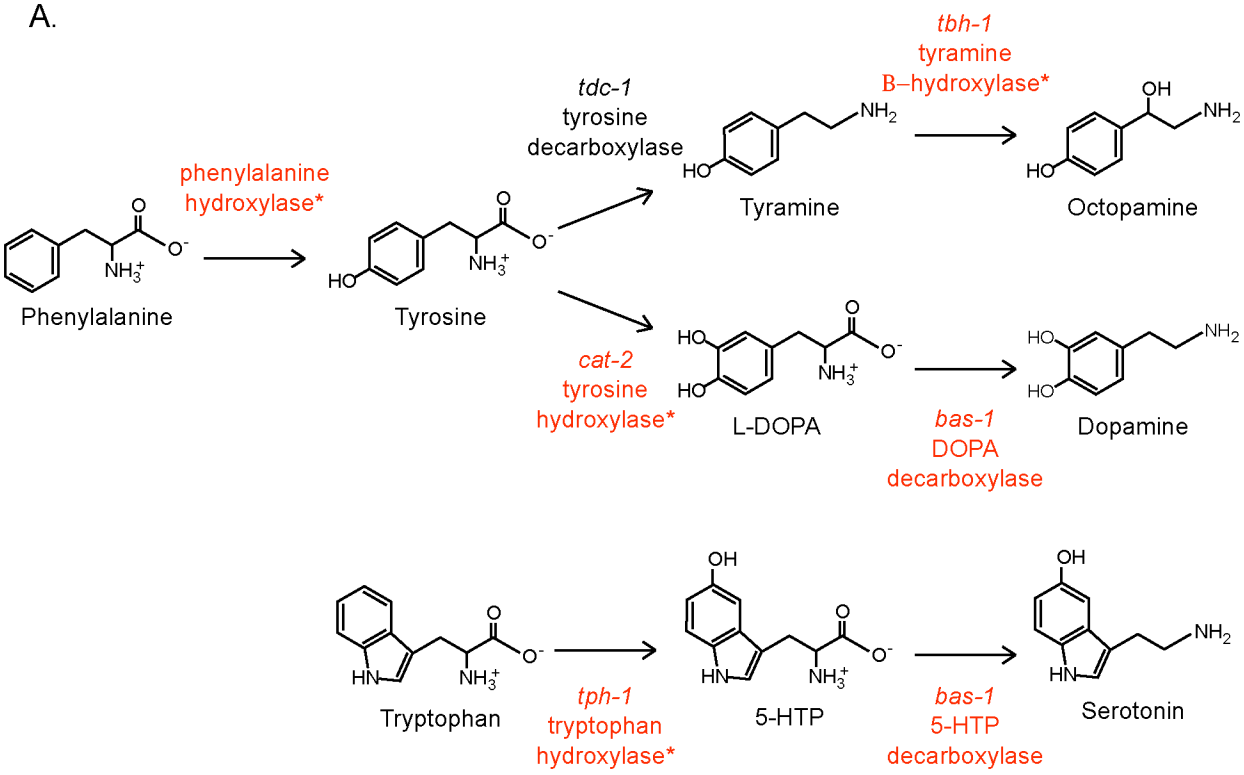

0.15
0.12
0.09
0.06
0.03
0.00
average speed (mm/s)
**
*
**
wild type
tph-1(n4622)
tph-1(mg280)
no bacteria
well-fed
food-deprived

Figure 3.3

The locomotion of the *bas-1;cat-4* mutant is altered with the addition of a mutation in *tph-1*. (A) *bas-1* encodes a decarboxylase while *cat-4* encodes a GTP cyclohydrolase. Both *bas-1* and *cat-4* are involved in biosynthetic pathways that make dopamine and serotonin. The pteridine cofactor produced by *cat-4* is also used by hydroxylase family members that are involved in the synthesis of tyrosine, octopamine, and other neurotransmitters. (B) Average velocities of wild-type, *bas-1(ad446);cat-4(e1141)*, and *bas-1(ad446);cat-4(e1141)tph-2(n4622)* animals under each condition. Error bars represent SEM. Asterisks indicate instances where the locomotion of the triple mutant is significantly different from the double mutant in the same condition ($p <$ *.05, **.001).

Figure 3.3

A.



*hydroxylase family member uses pteridine cofactor synthesized by GTP cyclohydrolase I *cat-4*

B.

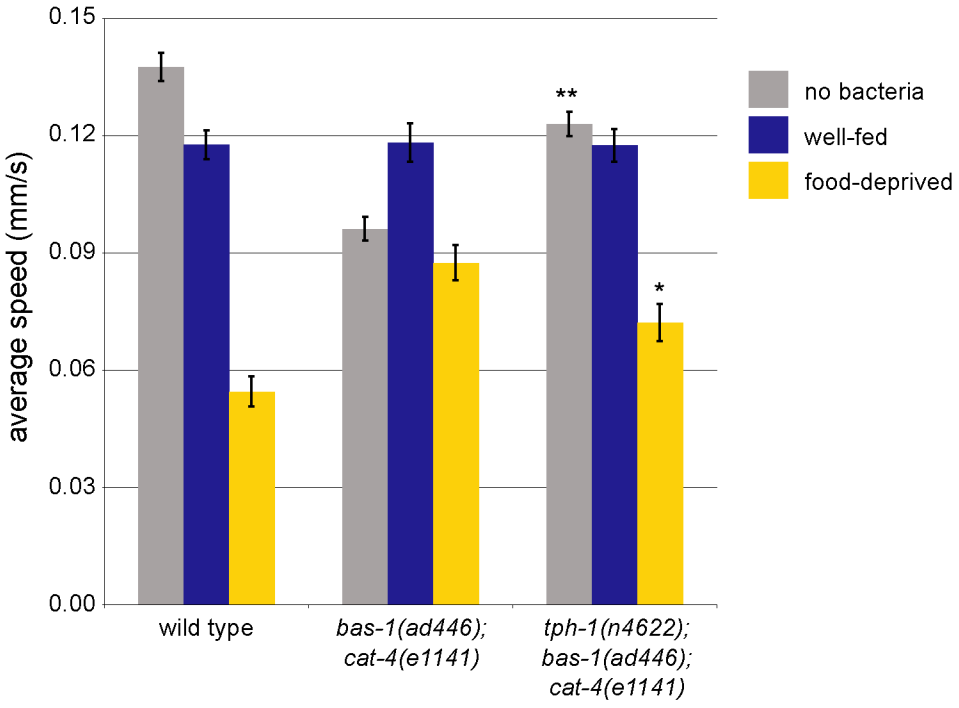
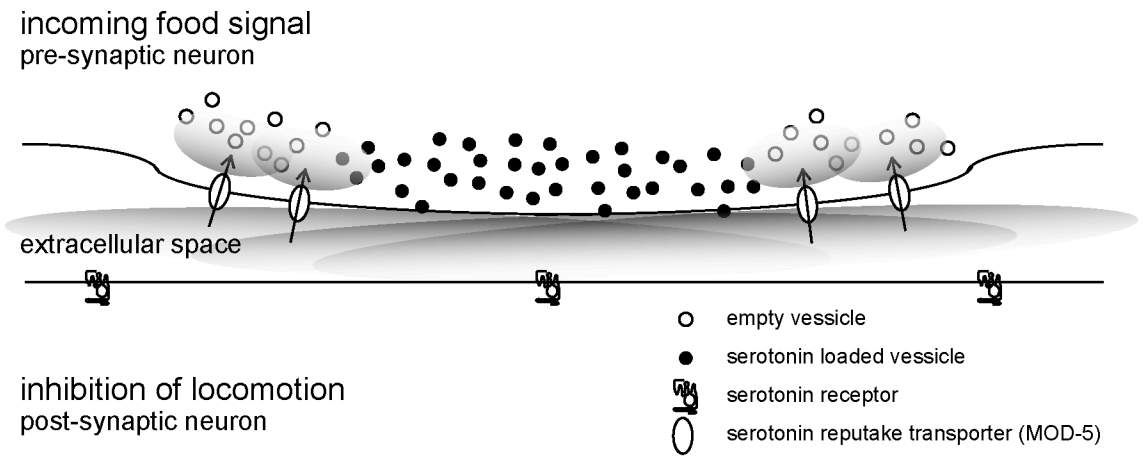


Figure 3.4

mod-5 mutants exhibit *tph-1* dependent reduced locomotion under all conditions. (A) Model: A bacterial food source stimulates serotonin release, which reduces the locomotion of animals. *mod-5* attenuates the serotonin-dependent slowing of animals on a bacterial lawn. (B) Average velocities of wild-type, *mod-5(n3314)*, *mod-5(n3314);tph-1(n4622)*, and *tph-1(n4622)* animals under each condition. Error bars represent SEM. Asterisks indicate instances where the locomotion of the *mod-5(n3314)* mutant is significantly different from wild-type locomotion in the same condition ($p <$ *.001).

Figure 3.4

A.


incoming food signal
pre-synaptic neuron
extracellular space
inhibition of locomotion
post-synaptic neuron
empty vessicle
serotonin loaded vessicle
serotonin receptor
serotonin reputake transporter (MOD-5)

B.

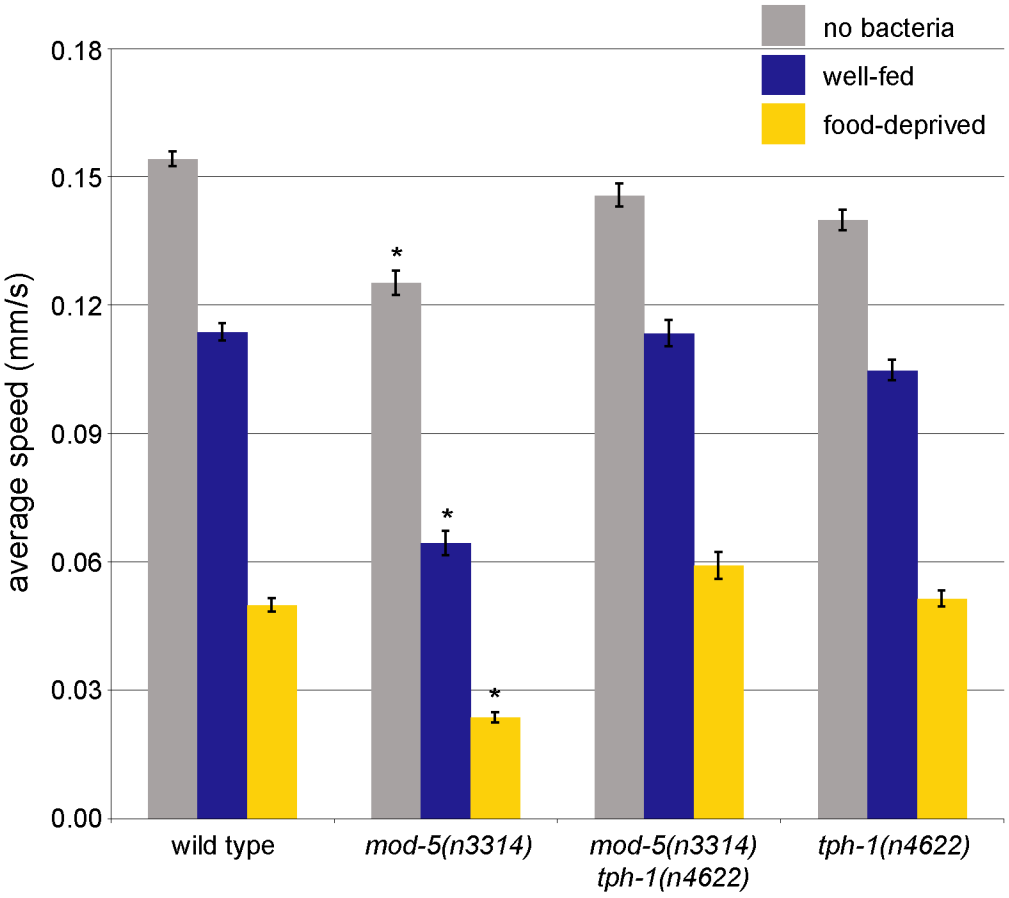

no bacteria
well-fed
food-deprived
average speed (mm/s)
0.18
0.15
0.12
0.09
0.06
0.03
0.00
*
*
*
wild type
mod-5(n3314)
mod-5(n3314) tph-1(n4622)
tph-1(n4622)

Figure 3.5

mod-1 mutants are resistant to immobilization by exogenous serotonin but do not have enhanced slowing defects. Mutants containing the *n4954* allele, present in the original *mod-1(n3034)* isolate, exhibit reduced enhanced slowing. (A) Average speed of wild-type animals, the original *mod-1* isolate, the outcrossed isolate with no *mod-1(n3034)* mutation, the outcrossed reisolated *mod-1(n3034)* allele, and *mod-1* deletion mutants *ok103* and *nr2043* in the off-bacteria and food-deprived on bacteria conditions. Error bars represent SEM. Asterisks indicate instances where the locomotion of the mutant is significantly different from wild-type locomotion in the same condition ($p < .001$). (B) Frequency distribution outlines of instantaneous speed recordings of food-deprived animals on a bacterial lawn. Outlines are plotted at 0.015 mm/second intervals. The fraction of wild-type and mutant animals that became immobilized in (C) 30 mM and (D) 50 mM serotonin in M9 buffer over the 20 second course of the assay.

Figure 3.5

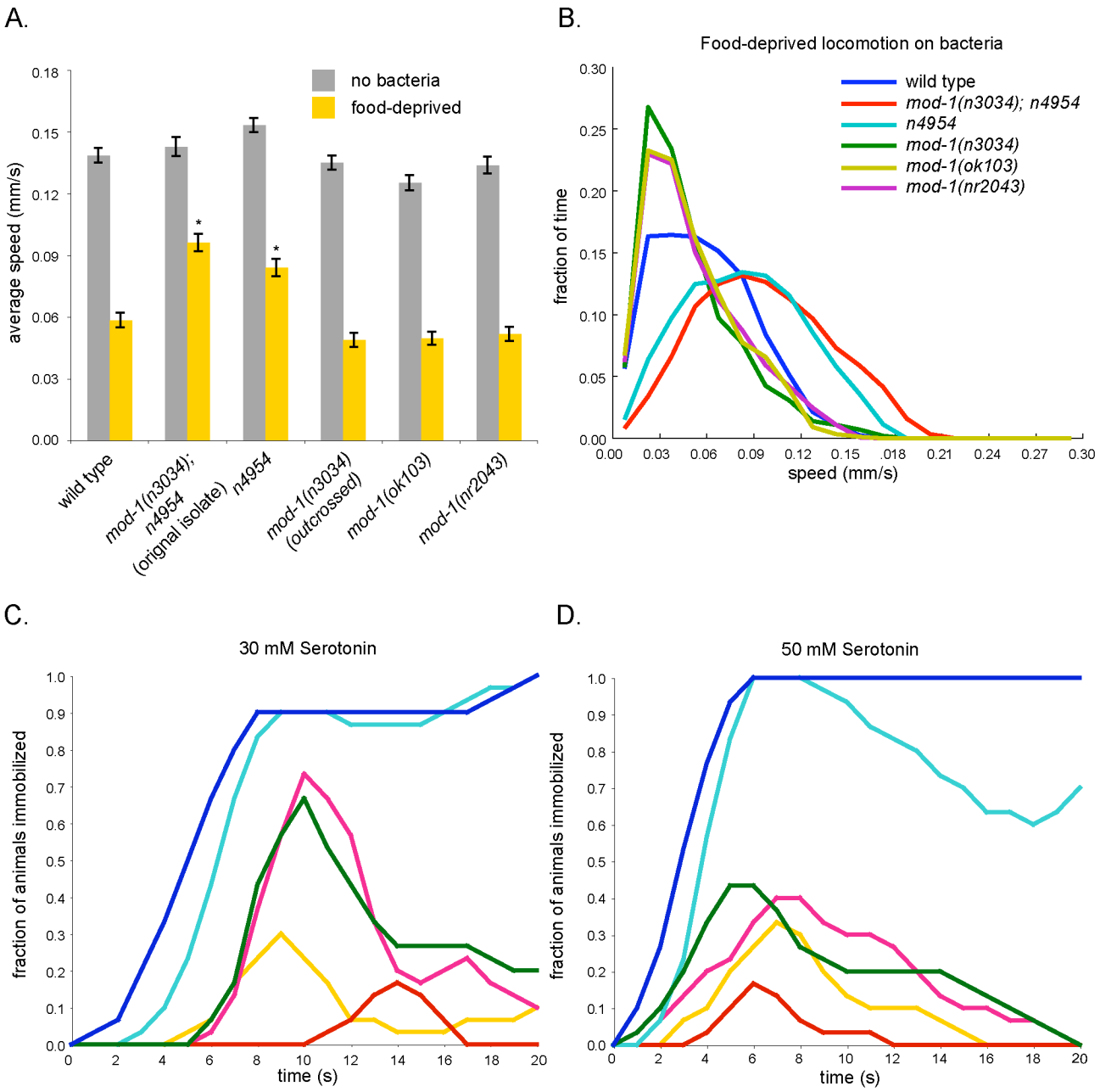
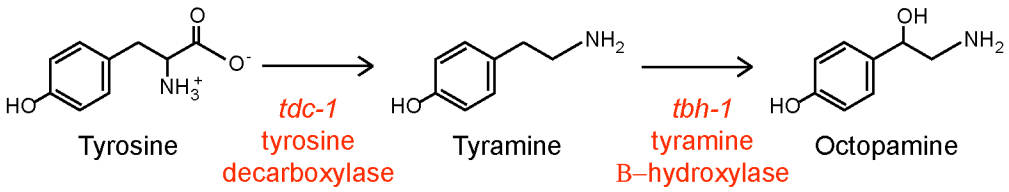

A.
no bacteria
food-deprived
average speed (mm/s)
0.18
0.15
0.12
0.09
0.06
0.03
0.00
wild type
mod-1(n3034); n4954 (orignal isolate)
n4954
mod-1(n3034) (outcrossed)
mod-1(ok103)
mod-1(nr2043)
B.
Food-deprived locomotion on bacteria
wild type
mod-1(n3034); n4954
n4954
mod-1(n3034)
mod-1(ok103)
mod-1(nr2043)
fraction of time
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0.00 0.03 0.06 0.09 0.12 0.15 .018 0.21 0.24 0.27 0.30
speed (mm/s)
C.
30 mM Serotonin
fraction of animals immobilized
1.0 0.9 0.8 0.7 0.6 0.5 0.4 0.3 0.2 0.1 0.0
0 2 4 6 8 10 12 14 16 18 20
time (s)
D.
50 mM Serotonin
fraction of animals immobilized
1.0 0.9 0.8 0.7 0.6 0.5 0.4 0.3 0.2 0.1 0.0
0 2 4 6 8 10 12 14 16 18 20
time (s)

Figure 3.6

Tyramine alone, or together with octopamine, stimulate *C. elegans* locomotion generally. (A) *tdc-1* encodes the *C. elegans* tyrosine decarboxylase enzyme involved in the synthesis of tyramine. *tbh-1* encodes the *C. elegans* tyramine β-hydroxylase involved in the synthesis of octopamine. (B) Average velocities of wild-type, *tdc-1(n3420)*, and *tbh-1(n3722)* animals under each condition. Error bars represent SEM. Asterisks indicate instances where the locomotion of the mutant is significantly different from wild-type locomotion in the same condition ($p <$ *.005, **.001).

Figure 3.6

A.


HO
O
O-
NH3+
Tyrosine
tdc-1
tyrosine
decarboxylase
HO
NH2
Tyramine
tbh-1
tyramine
B–hydroxylase
OH
HO
NH2
Octopamine

B.

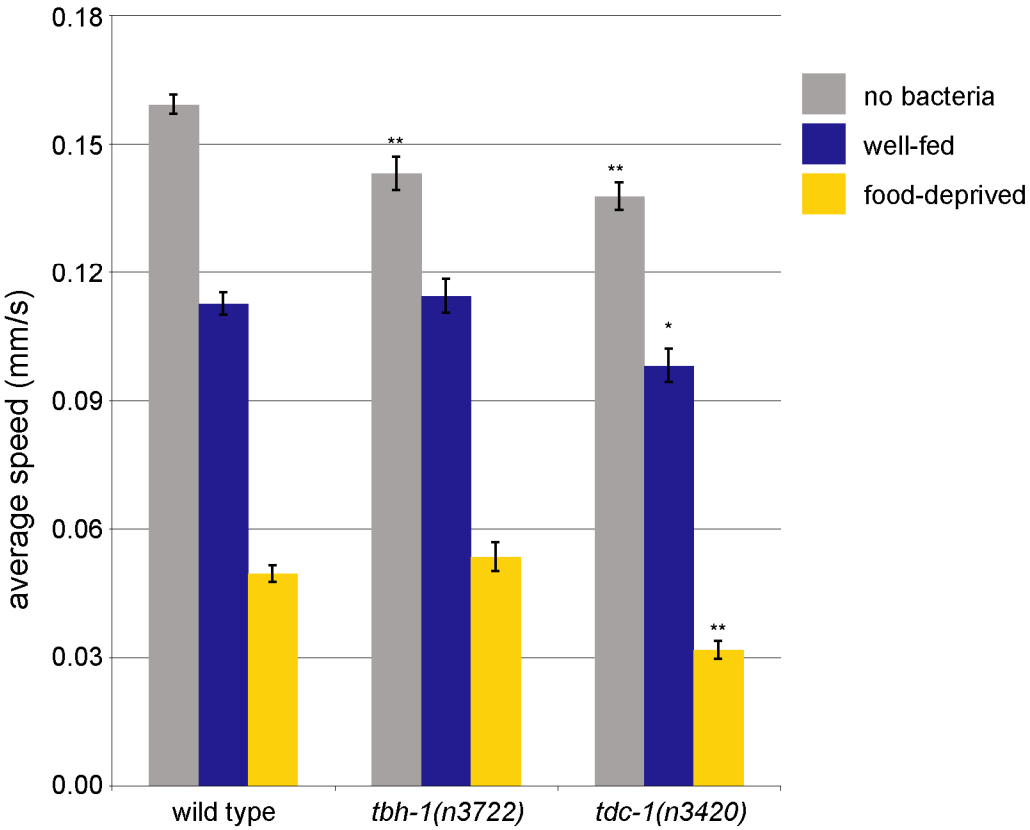

0.18
0.15
0.12
0.09
0.06
0.03
0.00
average speed (mm/s)
**
**
*
**
wild type
tbh-1(n3722)
tdc-1(n3420)
no bacteria
well-fed
food-deprived

Figure 3.7

cat-2(n4547) tph-1(n4622) tdc-1(n3420) triple mutants lacking dopamine, serotonin, octopamine, and tyramine exhibit slightly increased locomotion in the well-fed conditions but otherwise grossly normal locomotion. Average velocities of animals under each condition are presented. Error bars represent SEM. Asterisks indicate instances where the locomotion of the mutant is significantly different from wild-type locomotion in the same condition ($p < *0.001$).

Figure 3.7

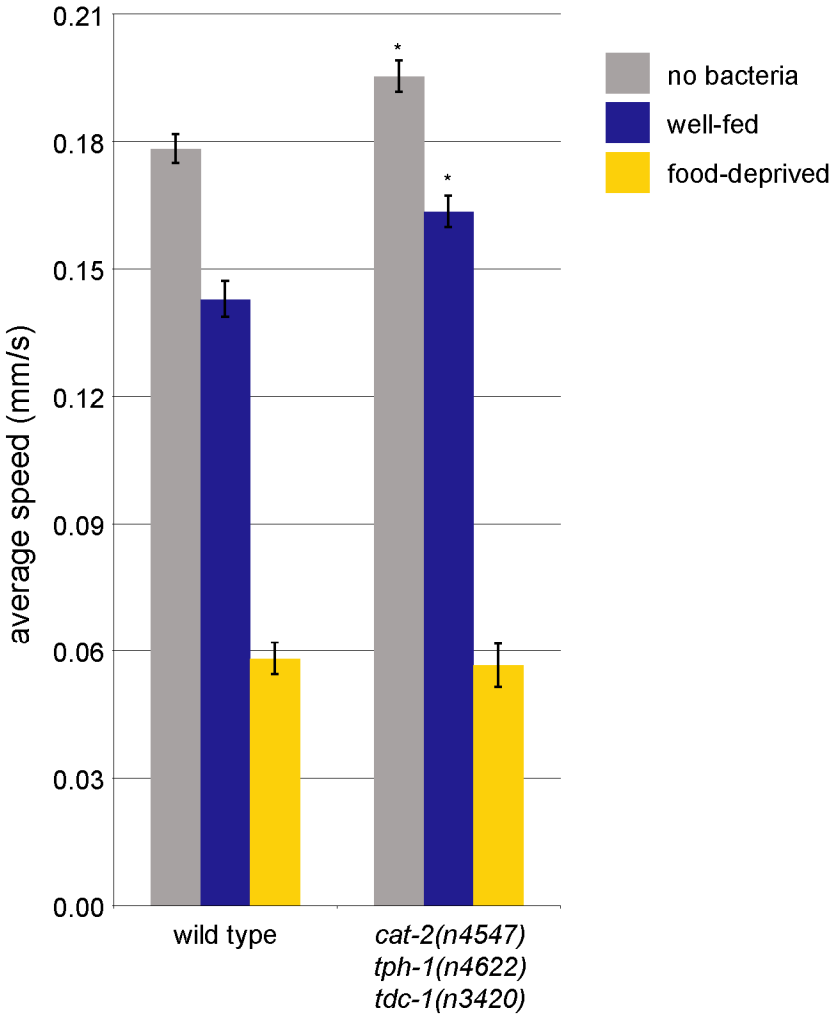

0.21
0.18
0.15
0.12
0.09
0.06
0.03
0.00
average speed (mm/s)
*
*
no bacteria
well-fed
food-deprived
wild type
cat-2(n4547)
tph-1(n4622)
tdc-1(n3420)

Figure 3.8

Mutation of *tph-1* results in reduced and increased locomotion in different mutant backgrounds. (A) In a *tdc-1(n3420)* or *cat-2(n4547) tdc-1(n3420)* mutant background, *tph-1(n4622)* mutation results in a general increase in locomotion rate. (B) In a *cat-2(n4547)* mutant background, *tph-1(n4622)* mutation results in a decrease in locomotion rate on a bacterial lawn. Error bars represent SEM. Asterisks indicate instances where the addition of a *tph-1(n4622)* mutation significantly alters the locomotion of animals in the same condition ($p <$ *.01, **.002, ***.001).

Figure 3.8

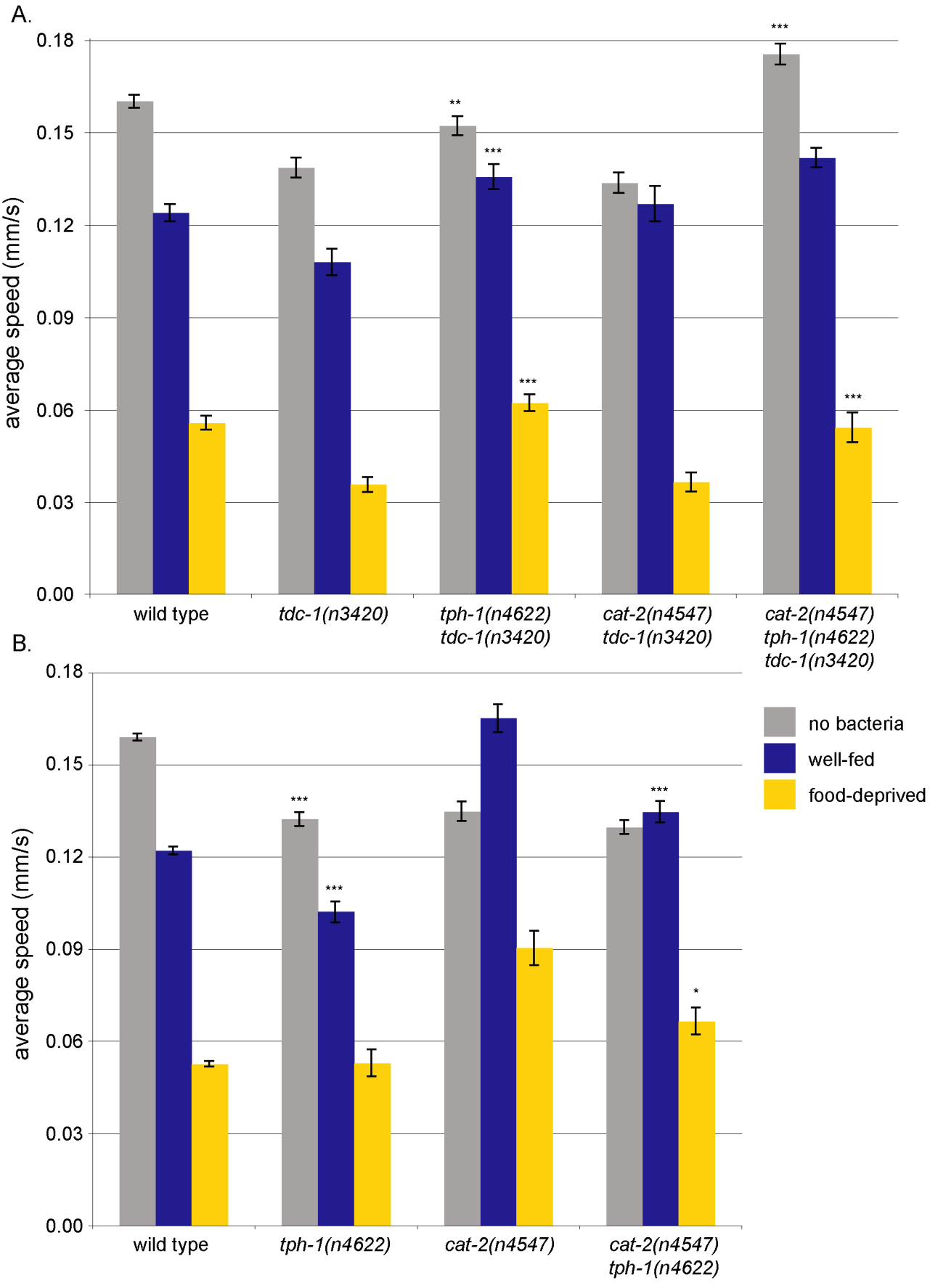

A.
average speed (mm/s)
0.18
0.15
0.12
0.09
0.06
0.03
0.00
**
***
***
***
***
wild type
tdc-1(n3420)
tph-1(n4622) tdc-1(n3420)
cat-2(n4547) tdc-1(n3420)
cat-2(n4547) tph-1(n4622) tdc-1(n3420)
B.
average speed (mm/s)
0.18
0.15
0.12
0.09
0.06
0.03
0.00
***
***
***
*
wild type
tph-1(n4622)
cat-2(n4547)
cat-2(n4547) tph-1(n4622)
no bacteria
well-fed
food-deprived

Figure 3.9

The locomotion stimulating activity of *tph-1* and *tdc-1* reciprocally depend on each other. (A) *tdc-1(n3420)* mutation causes a reduction in the locomotion of animals. In a *tph-1(n4622)* background, *tdc-1(n3420)* mutations no longer cause a reduction of locomotion on a bacterial lawn. (B) These data are consistent with a genetic pathway in which *tdc-1* inhibits *tph-1* which inhibits locomotion. (C) *tph-1(n4622)* mutation causes a reduction in the locomotion of animals on bacteria in a *cat-2(n4547)* mutant background. In a *cat-2(n4547) tdc-1(n3420)* background, *tph-1(n4622)* mutation no longer causes a reduction in locomotion but rather causes an increase in locomotion. (D) These data are consistent with a genetic pathway in which *tph-1* stimulates locomotion through *tdc-1*.

Figure 3.9

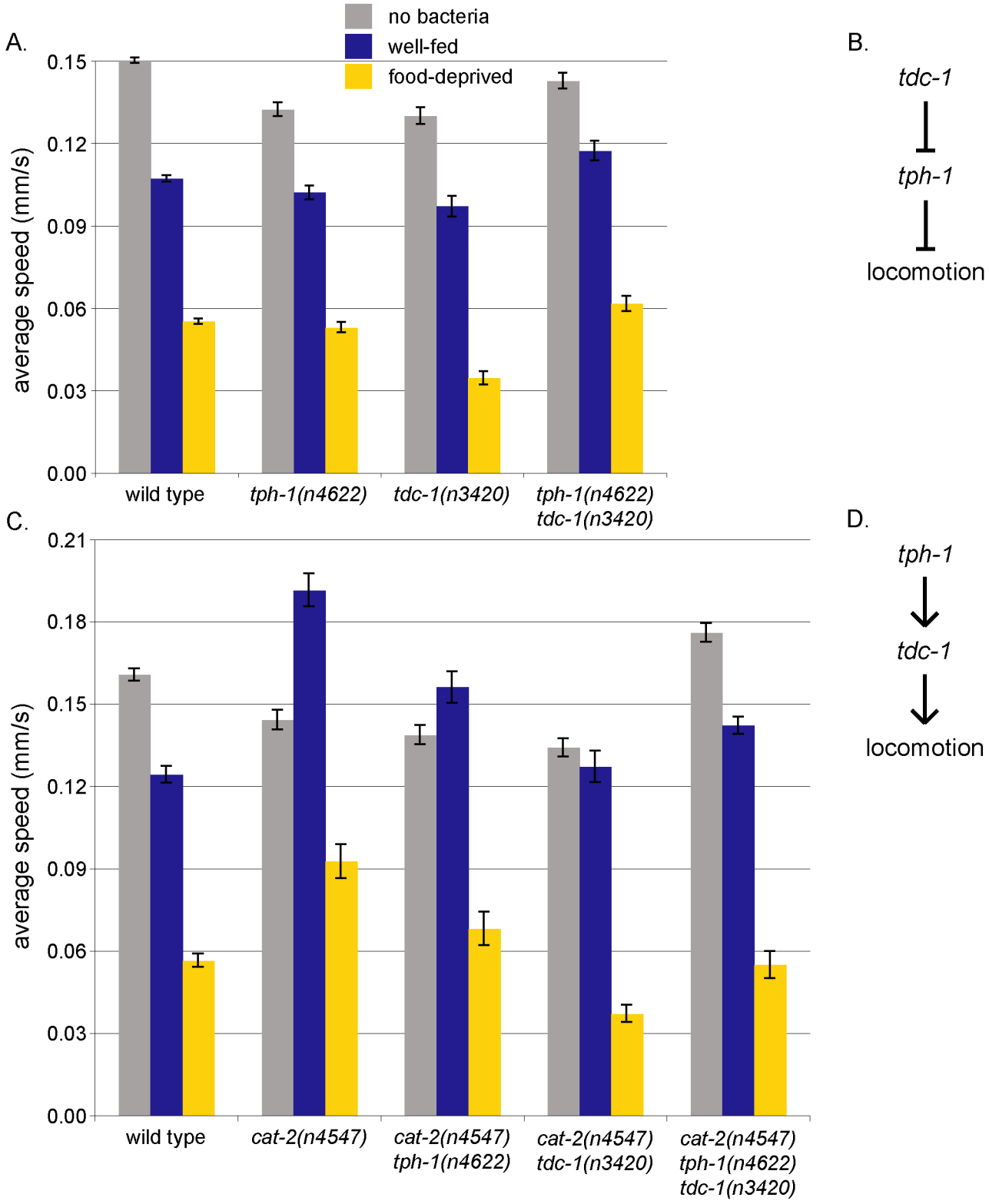
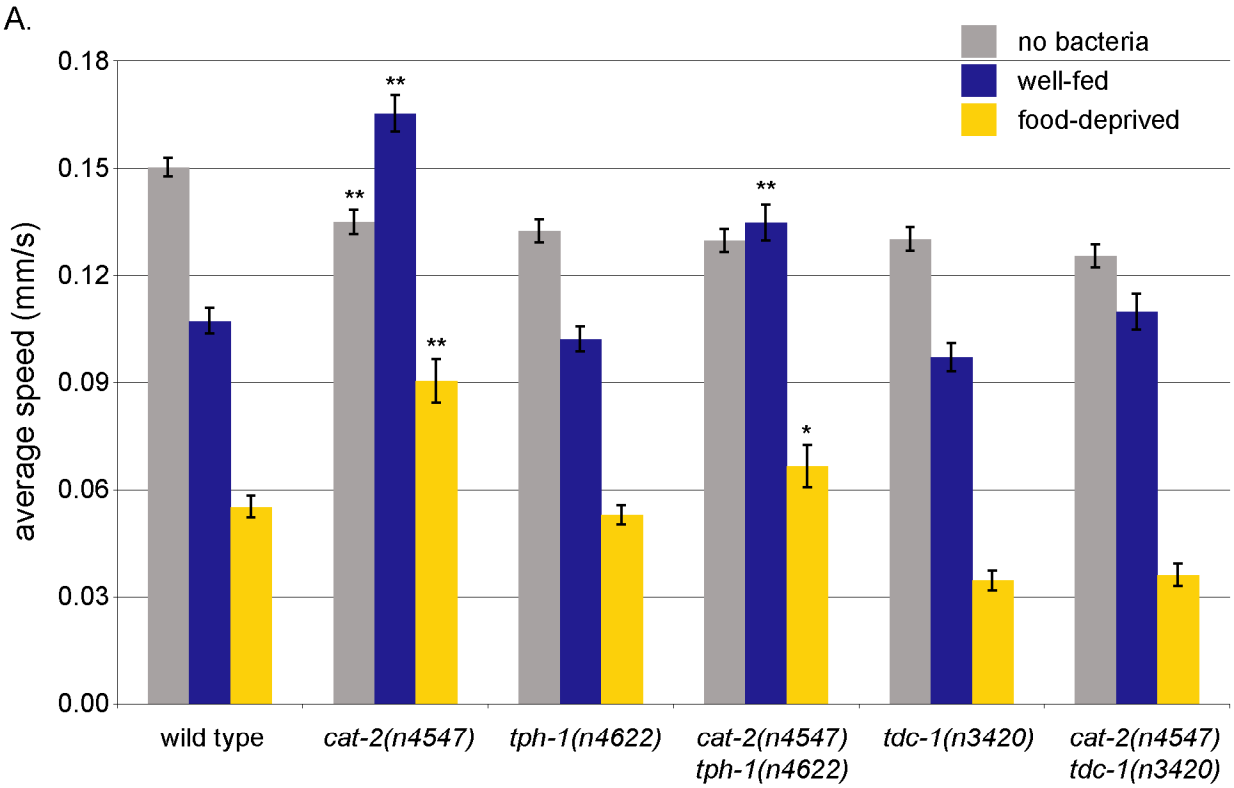

A.
no bacteria
well-fed
food-deprived
average speed (mm/s)
0.15
0.12
0.09
0.06
0.03
0.00
wild type
tph-1(n4622)
tdc-1(n3420)
tph-1(n4622) tdc-1(n3420)
B.
tdc-1
tph-1
locomotion
C.
average speed (mm/s)
0.21
0.18
0.15
0.12
0.09
0.06
0.03
0.00
wild type
cat-2(n4547)
cat-2(n4547) tph-1(n4622)
cat-2(n4547) tdc-1(n3420)
cat-2(n4547) tph-1(n4622) tdc-1(n3420)
D.
tph-1
tdc-1
locomotion

Figure 3.10

Mutation of *cat-2* results in *tdc-1* dependent increased locomotion. (A) *cat-2(n4547)* mutation causes an increase in the locomotion of animals. In a *tph-1(n4622)* background, *cat-2(n4547)* mutations are still able to cause a reduction of locomotion on a bacterial lawn, however in a *tdc-1(n3420)* mutant background addition of a *cat-2(n4547)* mutation no longer causes an increase in the locomotion of animals. Error bars represent SEM. Asterisks indicate instances where the addition of a *cat-2(n4547)* mutation significantly alters the locomotion of animals in the same condition ($p < *.05$, **.001). (B) These data are consistent with a genetic pathway in which *cat-2* inhibits locomotion through *tdc-1*.

Figure 3.10


A.
no bacteria
well-fed
food-deprived
average speed (mm/s)
0.18
0.15
0.12
0.09
0.06
0.03
0.00
**
**
**
**
*
wild type
cat-2(n4547)
tph-1(n4622)
cat-2(n4547) tph-1(n4622)
tdc-1(n3420)
cat-2(n4547) tdc-1(n3420)

B.

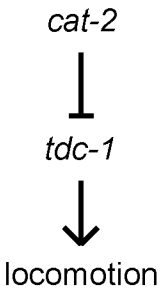
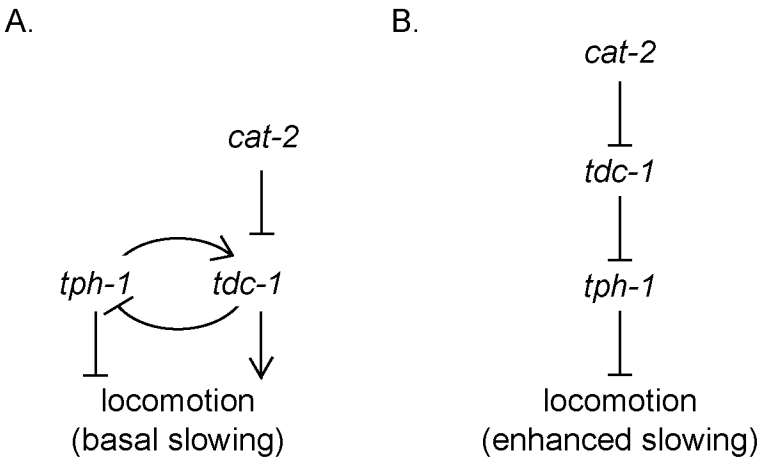

cat-2
tdc-1
locomotion

Figure 3.11

Genetic model that shows of *cat-2*, *tph-1*, and *tdc-1* in the regulation of *C. elegans* locomotion in the basal slowing and enhanced slowing conditions. (A) In the basal slowing condition *cat-2* inhibits locomotion through the regulation *tdc-1* which form a circuit of mutual regulation with *tph-1* (B) In the enhanced-slowing condition a linear pathway in which *cat-2* inhibits *tdc-1*, which inhibits *tph-1*, which inhibits locomotion, is supported.

Figure 3.11


A.
cat-2
tph-1
tdc-1
locomotion
(basal slowing)
B.
cat-2
tdc-1
tph-1
locomotion
(enhanced slowing)

CHAPTER 4

Dopamine signaling sets and maintains a precise rate of locomotion by *C. elegans*

Manuscript in preparation for publication

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Damon Clark wrote the Matlab code for all analysis. Data analysis methods were jointly conceived of by Damon and myself. I performed all experiments and was the primary author of this manuscript.

Summary

The nematode *Caenorhabditis elegans* detects a wide range of environmental and experiential cues that modulate its locomotor behavior. We developed an automated particle-tracking system that has greatly improved the resolution at which we can study *C. elegans* locomotion. Using this system, we observed that wild-type animals made fine adjustments to their speed to maintain constant rates of locomotion. Mutants defective in dopamine signaling made crude adjustments to their speeds that resulted in large fluctuations in their rates of locomotion. Additionally, these mutants exhibited both unusually high and low activity levels. These locomotion defects were reduced when tyramine and the related noradrenaline analog octopamine were eliminated. In human patients with Parkinson's Disease, a reduction in dopamine signaling can similarly lead to uncoordinated movements and abnormally high and low activity levels. We propose that in the *C. elegans* nervous system, dopamine signaling acts to monitor or integrate information about the current activity of the animal and that this information is then used to set and maintain an appropriate rate of locomotion. We further propose that a similar mechanism might exist in humans.

Introduction

Dopamine signaling modulates many mammalian neurobiological processes, including motor control, learning, and motivation (WISE 2004; SCHULTZ 2007). Abnormal dopamine function has been associated with a variety of disorders, including Parkinson's Disease, attention-deficit hyperactivity disorder (ADHD), and addiction (SOLANTO 2002; WISE 2004; HORNYKIEWICZ 2006). Parkinson's Disease is marked by deterioration of dopaminergic neurons in the brain, causing bradykinesia, rigidity, and resting tremors (TOLOSA *et al.* 2006). Administration of the dopamine precursor L-DOPA (Levodopa) is currently the most effective treatment for Parkinsonian symptoms (OLANOW *et al.* 2004).

While loss of dopaminergic neurons is most prominently associated with Parkinson's Disease, adrenergic signaling also appears to be altered in Parkinson's patients (ANTELMAN and CAGGIULA 1977; ROMMELFANGER and WEINSHENKER 2007). Substantial loss of noradrenergic neurons and autonomic homeostasis occur in Parkinson's patients with high frequency (AMINOFF and WILCOX 1971; GERMAN *et al.* 1992; POEWE 2006; DUBOW 2007). Noradrenaline is involved in sympathetic nervous function of the autonomic nervous system and is associated with the fight-or-flight response that is evoked in response to stress (CANNON 1927; LOEWY and SPYER 1990).

The *C. elegans* nervous system uses many neurotransmitters, neuromodulators, and signaling pathways that are evolutionarily conserved including dopamine (SULSTON *et al.* 1975; BARGMANN 1998). In this small nematode, dopamine controls a number of behaviors, including habituation to mechanical stimuli, foraging behavior, and food-dependent modulation of locomotion (SAWIN *et al.* 2000; CHASE *et al.* 2004; HILLS *et al.* 2004; SANYAL *et al.* 2004). Similar to the function of noradrenaline in humans, the invertebrate noradrenaline analog octopamine is thought to function in invertebrates as hormone triggered by environmental stress (ROEDER 1999). In *C. elegans*, octopamine modulates egg-laying and feeding behaviors, and both octopamine and the related biogenic amine tyramine are involved in the inhibition of food-dependent foraging behaviors (HORVITZ *et al.* 1982; ALKEMA *et al.* 2005).

C. elegans locomotion consists of coordinated dorsal-ventral muscle contractions that propel the nematode through its environment in a sinusoidal fashion (WHITE *et al.* 1976). *C. elegans* detects temperature, salts, soluble and volatile chemicals, oxygen, mechanical stimuli and other environmental cues that modulate its motor program as the animal seeks conditions favorable for growth and reproduction and avoids unfavorable conditions (WARD 1973; BRENNER 1974; HEDGECOCK and RUSSELL 1975; CULOTTI and RUSSELL 1978; BARGMANN and HORVITZ 1991; BARGMANN *et al.* 1993; SAWIN *et al.* 2000; GRAY *et al.* 2004; ZHANG *et al.* 2005). The behaviors of *C. elegans* are generated by a nervous system of only 302 neurons of known synaptic connectivity (WHITE *et al.* 1986).

Upon entering a bacterial lawn worms reduce their rates of locomotion (SAWIN *et al.* 2000). Food-deprived animals reduce their locomotion rate to a greater extent than do

well-fed animals in this food-rich environment. By contrast, food deprivation has very little effect on the locomotion of animals in the absence of a bacterial lawn. The reduced locomotion rates of well-fed and food-deprived animals on a bacterial lawn have been named the basal and enhanced slowing responses, respectively. Dopamine is required for the basal slowing response (SAWIN *et al.* 2000).

We have developed a locomotion tracking system that has enabled us to observe the individual locomotor behavior of multiple animals simultaneously with high spatial and temporal resolution. Using this system we have observed roles for dopamine in the regulation and coordination of *C. elegans* motor activity that might have parallel functions in regulating motor activity levels and coordinating movements in humans. We have also discovered a key role for tyramine and the related noradrenaline analog octopamine in the dopamine-dependent coordination of locomotor activity by *C. elegans*. Our genetic analysis defines interactions between dopamine and these noradrenaline-like signaling pathways in *C. elegans* that also might exist in humans.

Results

An automated tracking system enables the analysis of new aspects of C. elegans locomotor behavior

We developed an automated tracking system that determines the positions of animals on an assay plate at one sec intervals and assembles these position recordings into tracks. From these tracks, the instantaneous speed of every animal can be determined. This system preserves the integrity of each track, allowing the study of individual animals in a population (Figure 4.1A). This system also allows the determination of single-track parameters, such as average speed, variance of speed, frequency of adjustments to speed, and magnitude of accelerations and decelerations. This system can track at least 100 animals simultaneously.

The instantaneous speed recordings of a population of animals can be represented by histograms that show the fraction of time animals move at each given range of speeds.

These frequency distributions can be overlaid to compare how populations of animals move under different experimental conditions (Figure 4.1B). We found that wild-type *C. elegans* move at an average speed of 0.16 mm/sec on a solid agar surface in the absence of bacteria (Figure 4.1C).

As noted above, a bacterial food source induces a greater reduction of locomotion by food-deprived *C. elegans* than by well-fed animals (SAWIN *et al.* 2000). On a bacterial lawn, well-fed animals exhibited a 24% reduction in their rate of locomotion and food-deprived animals exhibited a 68% reduction in their rate of locomotion compared to their locomotion rate in the absence of bacteria (Figure 4.1C). These measurements are comparable to those obtained by manually counting the number of body bends an animal makes over a 20 sec interval using the scoring system described by Sawin et al. (SAWIN *et al.* 2000) Using this manual assay, we observed that on a bacterial lawn well-fed animals exhibited a 21% reduction in their locomotion rate, and food-deprived animals exhibited a 70% reduction in their rate of locomotion compared to their locomotion rate in the absence of bacteria (Figure 4.1D).

The variances of speeds of individual animals correlate with average locomotion rates regardless of feeding state

Since the locomotion rates of well-fed and food-deprived animals on a bacterial lawn overlap substantially, we attempted to identify other aspects of *C. elegans* locomotor behavior that might better distinguish between well-fed and food-deprived animals. We first examined the variances of locomotion rates of individual animals by calculating the standard deviations of the speed measurements of individual tracks. We also calculated each coefficient of variation, which is the standard deviation of speed measurements divided by the average speed of the animal. The coefficient of variation reflects the amount of variation in each track relative to average speed.

Although the standard deviation of individual tracks changed significantly as the average speed of the animal changed, the level of variation was dependent on the average speed and was not specifically affected by whether the animal had been food-deprived (Figure 4.1E). As the average speed of an animal increased, the instantaneous speed

measurements of the animal fluctuated less relative to average speed (Figure 4.1F). Feeding state therefore modulates the average speed of an animal but does not change the variability of the instantaneous speed of an individual animal independently of modulating average speed. In subsequent analysis of locomotion variability we therefore pooled our data for well-fed and food-deprived animals on bacteria to examine locomotion over a large range of average locomotion rates.

Dopamine-deficient mutants slow less than wild-type animals in response to a bacterial lawn

To analyze the role of dopamine in the modulation of *C. elegans* locomotion by a food source, we examined *cat-2* mutants. The gene *cat-2* encodes tyrosine hydroxylase that catalyzes the conversion of tyrosine to L-DOPA, the biosynthetic precursor of dopamine (NAGATSU *et al.* 1964). *cat-2* mutants have greatly reduced levels of dopamine (SULSTON *et al.* 1975; LINTS and EMMONS 1999). Previously it was reported that well-fed *cat-2* mutants failed to reduce their locomotion in response to a bacterial food source (SAWIN *et al.* 2000). We observed that in the well-fed state *cat-2* mutants not only failed to slow but actually increased their rate of locomotion on a bacterial food source, a finding that will be discussed below (Figure 4.2B). Additionally, we observed that *cat-2* animals exhibited less food-dependent slowing than did the wild type in the food-deprived state (Figure 4.2B). The two independently isolated *cat-2* mutants, *cat-2(e1112)* and *cat-2(n4547)*, behaved indistinguishably with respect to average rates of locomotion in all conditions tested (Figure 4.2B,4.7A).

Dopamine signaling is required to set the average speed and to restrict the range of speeds at which an individual animal travels

We noted that the instantaneous speeds of *cat-2* mutants on a bacterial lawn were more broadly distributed than the instantaneous speeds of wild-type animals in both the well-fed and food-deprived conditions (Figure 4.2A). To further define the nature of this abnormality, we first examined the average speeds of animals in each group. We found

that the average speeds of individual *cat-2* mutants were also more broadly distributed than were the average speeds of wild-type animals (Figure 4.2C). From these data we concluded that dopamine is required to precisely set the average activity level of wild-type animals.

We then compared the variations of the instantaneous speeds within individual wild-type and *cat-2* tracks. Our analysis of the locomotion of individual animals identified a second basis for the broad distribution of instantaneous speeds of *cat-2* mutants. Specifically, we found that when we examined individual animals moving at the same average speeds, *cat-2* mutants exhibited a greater range of instantaneous speeds than did wild-type animals. Overlaying multiple wild-type and *cat-2* speed traces with comparable average speeds illustrates that the locomotion rates of individual *cat-2* animals varied more than those of individual wild-type animals (Figure 4.2D). To quantify this difference we calculated the standard deviation of speed measurements within individual *cat-2* and wild-type tracks. The standard deviations of the instantaneous speeds of *cat-2* tracks were much larger than those of wild-type tracks (Figure 4.3A). These data show that dopamine is required to restrict the range of instantaneous speeds of individual wild-type animals.

Greater variation in the speeds of individual animals might explain why populations of *cat-2* mutants exhibit wider distributions of instantaneous speeds but would not explain why individual *cat-2* animals exhibit a wider range of average speeds. We therefore conclude that dopamine has a role in setting the average activity of animals and in restricting fluctuations in locomotion rate.

Dopamine-deficient mutants make unusually large adjustments to their speed

To investigate the increased fluctuations in *cat-2* locomotor activity, we quantified the number of accelerations and decelerations each animal made as well as the magnitude of accelerations and decelerations of each track. While *cat-2* mutants adjusted their speed approximately as frequently as did wild-type animals, the magnitude of their accelerations and decelerations were much greater than that of wild-type animals (Figure 3B,C). We interpret these results to indicate that *cat-2* mutants make larger than normal

adjustments to their speed and make these adjustments with approximately normal frequency.

Thus, dopamine functions to restrict the magnitude of the accelerations and decelerations made by an individual, explaining why individual *cat-2* animals exhibit a greater range of locomotion rates than do individual wild-type animals.

Dopamine modulates locomotion through the metabotropic D2-like dopamine receptor DOP-3 and $G_{\alpha o}$ protein GOA-1

Mutations in the metabotropic D2-like dopamine receptor *dop-3* and the $G_{\alpha o}$ protein *goa-1* cause locomotor hyperactivity and resistance to immobilization by exogenous dopamine (SAWIN *et al.* 2000; CHASE *et al.* 2004). We tested *dop-3* and *goa-1* mutants for *cat-2*-like locomotion defects and found that both mutants also made unusually large adjustments to their speed and exhibited a greater range of instantaneous speeds (Figure 4.4A-C).

Mutations in genes that encode other G protein-coupled biogenic amine receptors -- *dop-1(vs100)*, *dop-2(vs105)*, *dop-4(ok1321)*, *ser-1(ok345)*, *ser-2(pk1357)*, *ser-4(ok512)*, *tyra-3(ok325)*, *F14D12.6(ok371)*, and *T02E9.3(ok568)* (HAMDAN *et al.* 1999; REX and KOMUNIECKI 2002; CHASE *et al.* 2004; DEMPSEY *et al.* 2005; GUSTAFSON 2007) -- did not result in similar defects (data not shown). To test whether hyperactive locomotion is sufficient to cause higher variation in the instantaneous locomotion rates of individuals, we examined *npr-1* mutants, which are hyperactive on a bacterial lawn (DE BONO and BARGMANN 1998). Despite being hyperactive, *npr-1* individuals exhibited normal variation of instantaneous locomotion rates indicating that hyperactivity does not itself cause higher variation in individual locomotion rates (Figure 4.7A,B).

Treatment with exogenous dopamine restores normal modulation of locomotion to mutants defective in dopamine synthesis

Pretreatment of *cat-2* mutant animals with exogenous dopamine restored their ability to make fine adjustments to their speed and restrict the range of speeds at which

they travel (Figure 4.4D-F). These data show that reduced dopamine signaling in the adult worm causes the behavioral defects associated with *cat-2* mutation. Exogenous dopamine did not rescue this defect in *dop-3* and *goa-1* mutants, suggesting that these genes act in the postsynaptic reception and transduction of a dopamine signal, respectively (Figure 4.4D-F).

Mutations that attenuate tyramine and octopamine signaling suppress the defects associated with reduced dopamine levels

Tyramine is the biologically active precursor to the invertebrate noradrenaline analog octopamine (ALKEMA *et al.* 2005). Tyrosine is decarboxylated by tyrosine decarboxylase to make tyramine, which is then hydroxylated by tyramine β-hydroxylase to make octopamine. *C. elegans* tyrosine decarboxylase and tyramine β-hydroxylase are encoded by the genes *tdc-1* and *tbh-1*, respectively (ALKEMA *et al.* 2005). *tdc-1(n3420)* mutants lack both tyramine and octopamine, while *tbh-1(n3722)* mutants lack only octopamine (ALKEMA *et al.* 2005). We tested *tbh-1* and *tdc-1* mutants and found that while both mutants exhibited reduced locomotion rates in the absence of a bacterial lawn, only *tdc-1* mutants had reduced locomotion rates on a bacterial lawn (Figure 4.5A,C). Thus, we propose that tyramine alone or tyramine together with octopamine is involved in stimulating *C. elegans* locomotion on a bacterial lawn.

We found that the elevated locomotion of *cat-2* mutants was almost completely suppressed by elimination of *tdc-1* function (Figure 4.5C). Mutation of *tdc-1* also partially suppressed the abnormally large range of instantaneous locomotion rates and large accelerations of *cat-2* mutants (Figure 4.5D-F). *tdc-1* single mutants exhibited normal variation of individual track speed and normal magnitude of acceleration events (Figure 4.5D-F). We considered the possibility that suppression of locomotion rate might reduce higher fluctuations in speed. However, mutation of the serotonin reuptake transporter *mod-5(n3314)*, which slows *cat-2* mutants, did not suppress the greater fluctuations in speed (Figure 4.7A,C).

In short, we found that eliminating tyramine and octopamine suppressed both locomotion defects associated with reduced dopamine signaling in *C. elegans* suggesting

that tyramine and/or octopamine signaling act downstream of dopamine signaling in setting, and likely in maintaining, a consistent activity level in *C. elegans*.

Discussion

We have identified a new function for dopamine in the modulation of *C. elegans* locomotion. We found that in addition to the previously described requirement for dopamine in the food-dependent suppression of locomotion (SAWIN *et al.* 2000; CHASE *et al.* 2004), dopamine acts to precisely set the average activity level of animals and to restrict the magnitude of acceleration and deceleration events that allow individuals to maintain a relatively even rate of locomotion. The D2-like dopamine receptor DOP-3 and the $G_{\alpha o}$ protein GOA-1 likely function in the reception and transduction, respectively, of the dopaminergic signal that acts in these modulatory controls. We also found that treatment of dopamine-deficient mutants with exogenous dopamine completely restored the ability to finely coordinate locomotion. Finally, we found that the increased locomotor activity of *cat-2* mutants is dependent on tyramine and/or octopamine signaling.

An increase of dopamine release might be an aspect of reward signaling in C. elegans that reduces the locomotion of animals on a food source

Previously it was reported that *cat-2(e1112)* mutants exhibit reduced basal slowing (SAWIN 1996; SAWIN *et al.* 2000) but demonstrate normal (SAWIN *et al.* 2000) or slightly reduced (SAWIN 1996) enhanced slowing. We observed that *cat-2* mutants exhibit reduced slowing on a bacterial food source in both states, showing that dopamine signaling is involved in reducing the locomotion of *C. elegans* on a bacterial food source independently of feeding state. Food-deprivation is still able to modulate locomotion of *cat-2* mutants on a bacterial food source, in the absence of normal dopamine signaling, which shows that food-deprivation can modulate *C. elegans* locomotion independently of normal dopamine signaling.

Upon entering a bacterial food source, dopamine-deficient well-fed *cat-2* mutants not only failed to slow but actually increased their rate of locomotion, a response opposite to that of wild-type animals. While bacteria are a food source for *C. elegans*, bacteria can also be pathogenic and provide aversive stimuli (DE BONO *et al.* 2002; ZHANG *et al.* 2005). We suggest that *cat-2* mutants might have a reduced ability to respond to positive locomotion-reducing food-associated cues from a bacterial lawn but retain the ability to respond to negative cues that would increase the locomotion of the animal to facilitate its escape from a potentially hostile environment.

Studies of mammalian reward signaling have shown that when an animal encounters an unexpected improvement in conditions, a phasic increase in dopamine release occurs (MONTAGUE *et al.* 2004). Encountering a bacterial food source constitutes an improvement in conditions for *C. elegans*, and such an encounter causes the animal to reduce its rate of locomotion in a dopamine-dependent manner. Thus dopamine-dependent reward signaling might be a process that is evolutionarily conserved from nematodes to humans. We propose that food-associated cues might trigger the release of dopamine to suppress locomotion and increase the time worms spend in a food-rich environment, thus improving their chance of survival and reproduction (Figure 4.6).

Dopamine might also act in monitoring the animal's current locomotor activity to precisely set and maintain a steady speed

As an animal moves through its environment it continually encounters cues that can influence its activity. We propose that activity-elevating and activity-reducing cues establish an activity setpoint or target activity level. The current activity of an animal is monitored and compared to the setpoint and adjustments are made to the animal's activity to approach the setpoint. When the difference between the current speed and the setpoint exceeds a certain threshold, an acceleration or deceleration is initiated (Figure 4.6).

The only dopaminergic neurons in *C. elegans* are the two ADE, two PDE, and four CEP sensory neurons (SULSTON *et al.* 1975), all of which have been suggested to have a proprioceptive role, sensing deflections in the body of the animal when it is

moving (SAWIN 1996). We propose that in addition to reward signaling, dopamine is involved in monitoring the activity of the animal. Specifically, as an animal begins moving, some or all of these dopaminergic sensory neurons might release dopamine, allowing the animal to sense, transmit, and/or integrate information about how fast it is moving to adjust its locomotion rate properly (Figure 4.6).

We have shown that *cat-2* mutants exhibit greater variation both in average activity and in the instantaneous speeds of individual animals when compared to wild type. Additionally, *cat-2* mutants exhibit larger acceleration and deceleration events, which lead to erratic locomotion. A reduced ability of an individual animal to integrate information about how fast it is moving might cause a greater variability in both average activity levels and instantaneous speeds, because the animal would have a reduced ability to detect when it is traveling too quickly or too slowly. Such a deficit might also result in greater acceleration and deceleration events, since an acceleration or deceleration event would terminate only when the animal has accelerated or slowed to an acceptable speed, the detection of which would be impaired.

Dopamine deficient *cat-2* mutants exhibit increased activity and large fluctuations in the locomotion rate, both of which are suppressed by the removal of noradrenergic-like signaling through mutation of *tdc-1*. We therefore place octopamine and tyramine signaling downstream of both reward and coordination aspects of dopamine signaling in our model (Figure 4.6).

Reduced dopamine signaling leads to improper regulation of activity levels and coordination of locomotion in both C. elegans and humans

Parkinson's Disease results from a reduction of dopamine signaling in the central nervous system and is characterized by bradykinesia, rigidity, and resting tremors (TOLOSA *et al.* 2006). Other symptoms induce orthostatic hypotension, urinary urgency and incontinence, constipation, sexual dysfunction, and sialorrhea, which indicate both sympathetic and parasympathetic dysfunction and suggest a role for dopamine in maintaining homeostatic balance in the autonomic nervous system (AMINOFF and WILCOX 1971; POEWE 2006; DUBOW 2007). Thus Parkinsonian symptoms appear to

reflect a reduced ability to maintain autonomic homeostasis and properly regulate motor activity levels.

Bradykinesia and rigidity appear to reflect both extremely reduced and increased stimulation of motor activity, respectively. Similar to Parkinson's patients, *C. elegans* mutants with attenuated dopamine signaling have defects in regulating motor activity levels. Dopamine-deficient *cat-2* mutants frequently exhibited activity levels at the outer range of normal wild-type activity. The hyperactivity of *cat-2* mutants was significantly suppressed when the noradrenaline-like neuromodulators tyramine and octopamine were also removed. Since tyramine and octopamine appear to be involved in the stimulation of *C. elegans* locomotion, the elevated activity of *cat-2* mutants is likely caused by increased activity of these signaling molecules that increase *C. elegans* response to locomotion stimulating inputs. This finding suggests that dopamine antagonizes adrenergic-like signaling in *C. elegans*.

Abnormal adrenergic signaling in Parkinson's patients has been noted for many years (ROMMELFANGER and WEINSHENKER 2007). One early model proposed that the loss of dopamine signaling leads to excess adrenergic signaling and that a compensatory mechanism leads to a reduction of adrenergic neurons in the brains of Parkinson's patients (ANTELMAN and CAGGIULA 1977; GERMAN *et al.* 1992). By analogy, our results in *C. elegans* also suggest that dopamine might have a conserved function in antagonizing adrenergic activity in humans. Specifically, we propose that reduced dopamine signaling might lead to an increase in adrenergic activity causing autonomic imbalance of Parkinson's patients, which might lead to extreme motor inactivity and activity like bradykinesia and rigidity.

The motor coordination defects of Parkinson's patients also include festinating or uneven gait and postural instability, which appear to reflect an inability to make fine adjustments to motor activity. Earlier we proposed that dopamine facilitates the detection or integration of information about peripheral locomotor activity that is required to make fine adjustments to locomotor activity in *C. elegans*. We propose that impaired dopamine signaling in humans might lead to festinating or uneven gait and postural instability by a similar mechanism.

Studies of the role of dopamine in C. elegans locomotor behavior might lead to insights concerning Parkinson's Disease

Administration of the dopamine precursor L-DOPA (levodopa) is the most effective treatment for Parkinson's symptoms (STERN 1997; OLANOW *et al.* 2004). We demonstrated that pre-incubation of animals with exogenous dopamine was able to restore normal locomotion to *C. elegans* mutants with reduced dopamine levels. Metabotropic dopamine receptor agonists (bromocriptine, apomorphine, pramipexole, ropinirole, rotigotine) constitute a second family of Parkinson's therapies (TULLOCH 1997; WATTS 1997). We have shown that mutation of a metabotropic D2 dopamine receptor homologue causes the same modulation defects associated with attenuated dopamine levels in *C. elegans*.

The molecular conservation between *C. elegans* and humans with respect to dopaminergic pathways that affect coordinated locomotion suggests that future studies of *C. elegans* might provide insights into Parkinson's Disease. The identification of genes and pathways that function either in or with dopamine signaling to set or adjust *C. elegans* locomotor activity might define new targets for Parkinsonian therapies. Similarly, studying the effects of current Parkinson's therapies on *C. elegans* might provide insight into how these drugs act both in reducing the symptoms of Parkinson's Disease and in causing undesirable side effects.

Methods

Strains

Caenorhabditis elegans strains were cultivated on NGM agar at 20°C as described by Brenner (1974), except that worms were grown on *E. coli* strain HB101 as a food source. The wild-type *C. elegans* strain used was N2. Other strains used in this paper are: AQ866 *ser-4(ok521)*, CB1112 *cat-2(e1112)*, CX4148 *npr-1(ky13)*, DA1814 *ser-1(ok345)*, LX645 *dop-1(vs100)*, LX702 *dop-2(vs105)*, LX703 *dop-3(vs106)*, MT2426

goa-1(n1134), MT9772 *mod-5(n3314)*, MT10661 *tdc-1(n3420)*, MT11374 *tbh-1(n3722)*, MT13710 *mod-5(n3314); cat-2(e1112)*, MT15620 *cat-2(n4547)*, MT16044 *cat-2(n4547) tdc-1(n3420)*, OH313 *ser-2(pk1357)*, RB785 *T02E9.3(ok568)*, RB1254 *dop-4(ok1321)*, VC125 *tyra-3(ok325)*, VC224 *F14D12.6(ok371)*. MT15620 *cat-2(n4547)* is a newly isolated strain that contains a 1010bp deletion of the *cat-2* locus that removes sequences 3' to ATGTGAAGTCACACCTGTCT and 5' to ATCATTTTGAAAATCCGACC.

Locomotion Assay

Assays were conducted according to Sawin et al. (2000) with the following modifications. Assay plates containing bacteria were made by spreading a drop of saturated HB101 culture using the smooth bottom of a small glass culture tube into the shape of a ring. Assay plates containing no bacteria or with a ring-shaped bacterial lawn were examined, and only those with minimal surface imperfections and a textured non-glassy-appearing bacterial lawn were used for locomotion assays. Assays were performed in a temperature controlled room at 22°C. The locomotion of 20-25 animals was recorded in each assay. Recording of locomotion was scored by an automated locomotion tracking system and began 5 min after animals were transferred to assay plates and lasted for 2 min.

We used a Sony XCD-X710 firewire camera at 1024x768 resolution to capture high-contrast dark-field images of our assay plates illuminated from the side by a ring of LEDs. Images were acquired at 1 Hz. The centers of mass (centroids) of all worms were found and individual position recordings were assembled into tracks from centroid positions using the methods outlined by Clark et al. (2007). Animal speeds were calculated by dividing the path distance an animal traveled by the time it took to travel that path. Each speed recording was calculated by averaging 5 speed measurements observed during a 5 sec sliding window to minimize the noise in centroid displacements. Such instantaneous speed measurements varied from 0 to roughly 0.3 mm per sec.

Dopamine rescue experiments were repeated on four different days, while all other experiments were repeated on a minimum of seven different days, pooled, and evaluated together. Data for a paired wild-type control were collected on each assay day.

Drug Pretreatment

A fresh solution of 50 mM dopamine hydrochloride (Sigma) in M9 buffer (WOOD 1988) was prepared, and 400 µl of this solution was immediately added to a standard plate containing approximately 10 ml NGM agar seeded with a bacterial lawn. Plates were allowed to dry for 1 hr. Control plates were made by adding 400 µl M9 to standard plates containing approximately 10 ml NGM agar seeded with a bacterial lawn. Animals were incubated on dopamine-containing plates or control plates for 4-6 hr prior to assay. Assay plates and food-deprivation plates did not contain dopamine.

Data Analysis

The average speed of a population was calculated to be the mean of all instantaneous speeds calculated for all animals tested. T values were determined using a student's t-test for comparing the means of two samples of unequal size. P-values were determined from a two-tailed student's t-distribution.

To analyze the variation in speed recordings within individual tracks, we first determined the average speed of each track and separated tracks into bins by average speed. The standard deviation of the intra-track speed recordings was then determined for each track. Individual standard deviation calculations were weighted linearly with respect to track length, and the average standard deviation for each bin was determined from these weighted calculations.

The frequency of acceleration was analyzed by first dividing tracks into bins by average speed. Instantaneous acceleration calculations were determined from speed measurements. An acceleration event was defined as a change in speed having a magnitude exceeding 0.006 mm per sec per sec (approximately 20% of the maximal wild-type acceleration). This threshold is the point at which non-moving animals did not exhibit high frequency of acceleration presumably due to small head movements. The number of acceleration events per sec was calculated for each track. Each calculation

was then weighted linearly with respect to track length, and the average number of acceleration events per sec was determined from these weighted calculations.

Magnitude of accelerations was calculated as the root mean square (RMS) of acceleration over time. The magnitude of accelerations was analyzed by first dividing tracks into bins by average speed. The RMS of accelerations was calculated for each track and was then weighted linearly with respect to track length and used to compute an average RMS acceleration for each bin.

To compare the average locomotion rates for mutants assayed on different days, we calibrated our results using paired wild-type controls collected on every assay day (Figure 4.5C,4.7A). First wild-type control data from all days were pooled to create a master wild-type dataset. A paired wild-type dataset was then created for each mutant, by pooling the wild-type control data for each day that specific mutant was assayed. To standardize data to the master control, we then multiplied the average speed of each mutant in each condition (off-food, well-fed, food-deprived) by the ratio between the master wild-type control and paired wild-type control (value Master / value Paired) for each condition. We found that this method most successfully maintained the integrity of the relationships between mutants when compared to data collected completely in parallel.

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Figures

Figure 4.1

Observations of wild-type *C. elegans* locomotion obtained using an automated locomotion tracking system. (A) A speed recording of an individual worm recorded by our tracking system. (B) Frequency distributions of instantaneous speed recordings of animals tested in the off-bacteria, well-fed on bacteria, and food-deprived on bacteria conditions. Each bin is 0.006 mm/sec wide. (C) Average speeds of animals tested in each condition as measured by the automated tracking system. Error bars represent SEM. (D) Average speeds of animals as measured by manually counting body bends over 20 sec intervals. Error bars represent SEM. (E) The average standard deviations of speed measurements within individual tracks are shown for well-fed and food-deprived animals. Tracks were first grouped by average speed. Each bin is 0.0075 mm/sec wide. Error bars represent SEM. (F) The average coefficients of variation (standard deviation / average track speed) of speed measurements within individual tracks are shown for well-fed and food-deprived animals. Tracks were first grouped by average speed. Each bin is 0.0075 mm/sec wide. Error bars represent SEM. (B,C,E,F: n = 1510 off-food, 1174 well-fed, 967 food-deprived animal tracks, mean track length was 53 sec in the automated assay; D: n = 528 off-food, 508 well-fed, 642 food-deprived animals assayed in the manual assay).

Figure 4.1

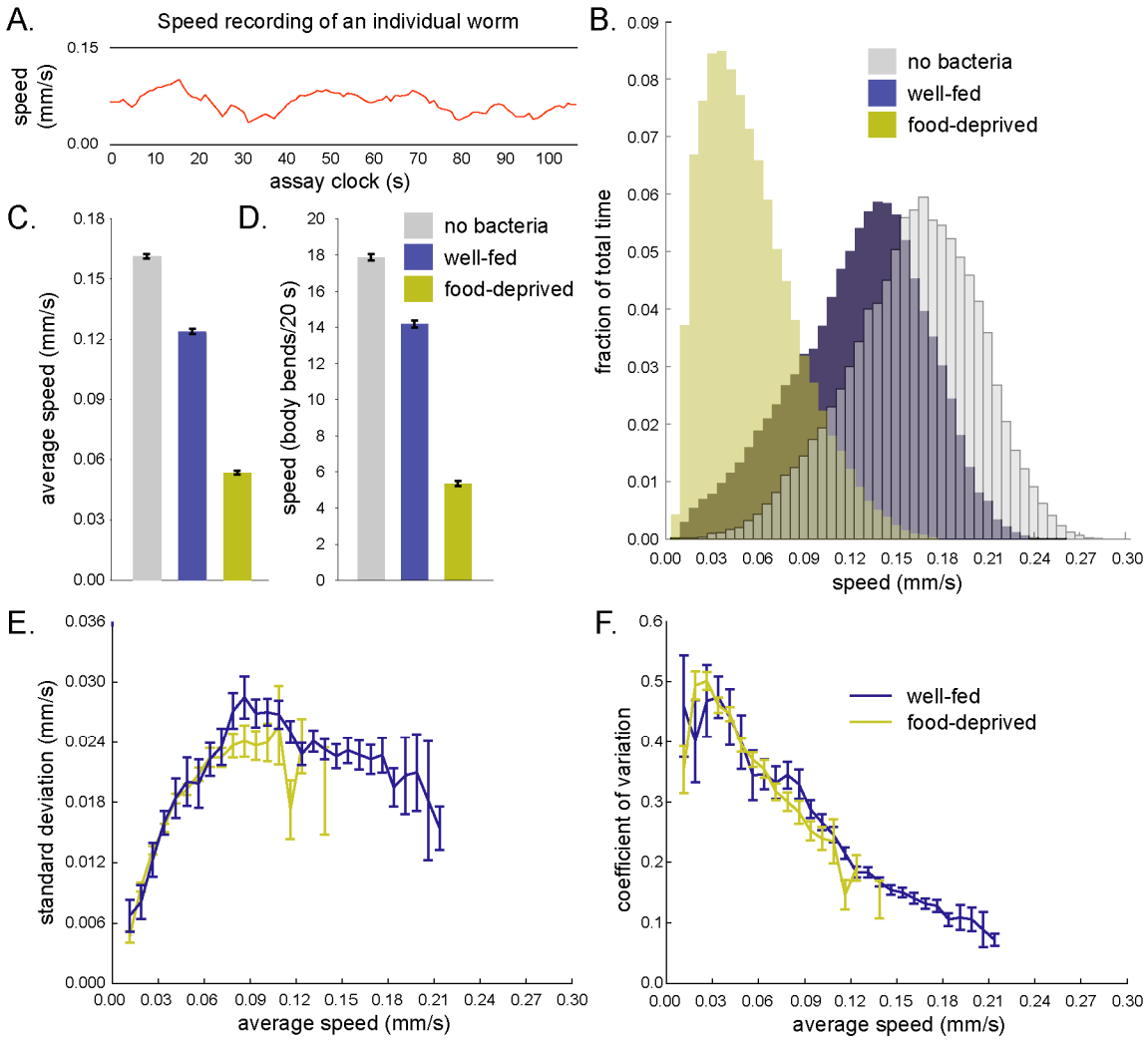

A.
Speed recording of an individual worm
speed (mm/s)
assay clock (s)
B.
no bacteria
well-fed
food-deprived
fraction of total time
speed (mm/s)
C.
average speed (mm/s)
D.
speed (body bends/20 s)
E.
standard deviation (mm/s)
average speed (mm/s)
F.
coefficient of variation
average speed (mm/s)

Figure 4.2

The locomotion rates of *cat-2* mutants on bacteria were elevated and more variable compared to the locomotion rates of wild-type animals. (A) Frequency distributions of instantaneous speed recordings of *cat-2(n4547)* mutants tested in the off-bacteria, well-fed on bacteria, and food-deprived on bacteria conditions. Each bin is 0.006 mm/sec wide. Wild-type frequency distribution outlines are overlaid for comparison. The standard deviations of instantaneous speed recordings in the no bacteria, well-fed, and food-deprived conditions were 0.036, 0.038, and 0.031 mm/sec for wild-type animals and 0.047, 0.057, and 0.066 mm/sec for *cat-2* mutants. (B) Average speeds of wild-type and *cat-2(n4547)* animals tested in each condition. Error bars represent SEM. (C) Frequency distributions of the average speeds of individual wild-type and *cat-2(n4547)* animals in the well-fed on bacteria and food-deprived on bacteria conditions. The standard deviations of average speeds of individual animals in the well-fed and food-deprived conditions were 0.032 and 0.027 mm/sec for wild-type animals and 0.049, and 0.057 mm/sec for *cat-2* mutants. (D) The 15 longest wild-type and *cat-2(n4547)* speed traces with average velocities of 0-0.06 mm/sec and 0.12-0.18 mm/sec were overlaid. (A-C: wild-type, n = 203 off-bacteria, 151 well-fed on bacteria, 135 food-deprived on bacteria animal tracks, *cat-2*, n = 216, 160, 139)

Figure 4.2

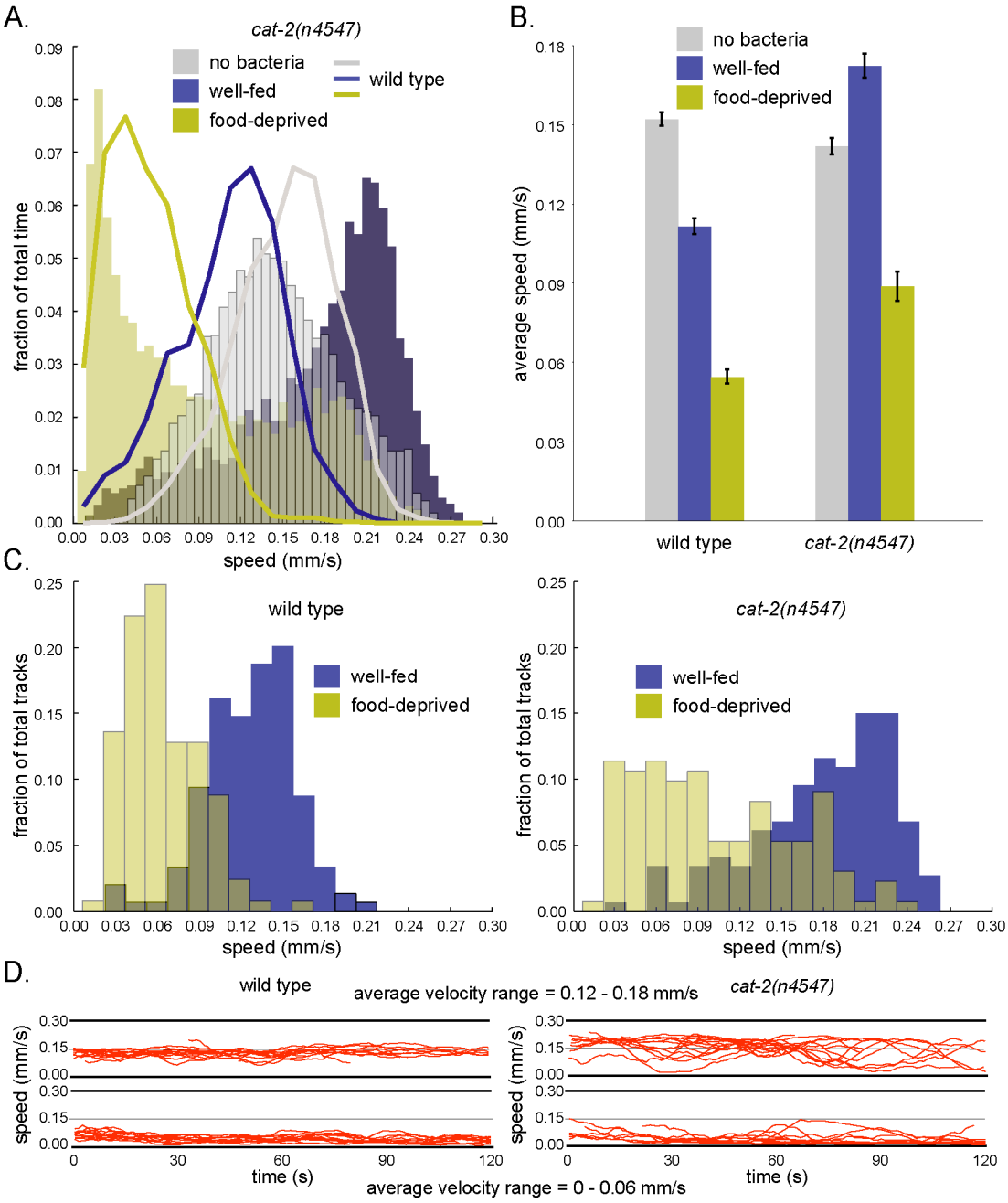

A.
cat-2(n4547)
no bacteria
well-fed
food-deprived
wild type
fraction of total time
speed (mm/s)
B.
no bacteria
well-fed
food-deprived
average speed (mm/s)
wild type
cat-2(n4547)
C.
wild type
well-fed
food-deprived
fraction of total tracks
speed (mm/s)
cat-2(n4547)
well-fed
food-deprived
fraction of total tracks
speed (mm/s)
D.
wild type
average velocity range = 0.12 - 0.18 mm/s
cat-2(n4547)
speed (mm/s)
time (s)
average velocity range = 0 - 0.06 mm/s
speed (mm/s)
time (s)

Figure 4.3

cat-2 mutants accelerated more than wild-type animals but adjusted their speed with approximately normal frequency resulting in greater fluctuations in the speed measurements within individual tracks. The average (A) standard deviations of speed measurements within individual tracks, (B) frequency of acceleration events (see Methods), and (C) magnitude (root mean square) of accelerations of animals are shown for wild-type and *cat-2(n4547)* animals on a bacterial lawn. Tracks were first grouped by average speed. Error bars represent SEM. (wild type, n = 286 tracks, *cat-2(n4547)*, n = 299 tracks)

Figure 4.3

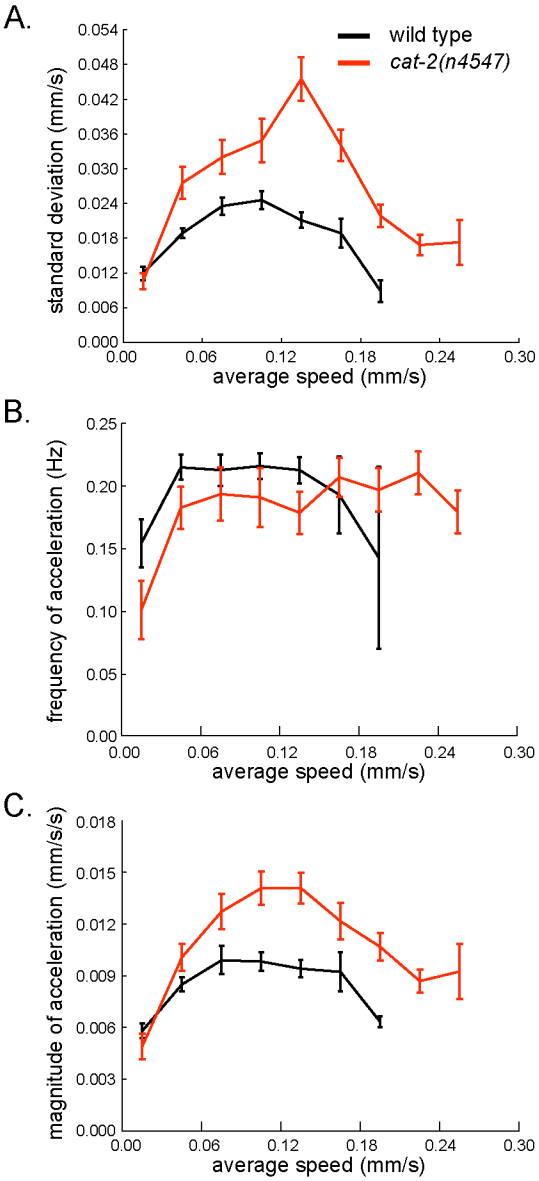

A.
wild type
cat-2(n4547)
standard deviation (mm/s)
0.054
0.048
0.042
0.036
0.030
0.024
0.018
0.012
0.006
0.000
0.00
0.06
0.12
0.18
0.24
0.30
average speed (mm/s)
B.
frequency of acceleration (Hz)
0.25
0.20
0.15
0.10
0.05
0.00
average speed (mm/s)
C.
magnitude of acceleration (mm/s/s)
0.018
0.015
0.012
0.009
0.006
0.003
0.000
average speed (mm/s)

Figure 4.4

Pre-treatment with exogenous dopamine restores normal locomotion to *cat-2* mutants but not to *dop-3* or *goa-1* mutants. The average (A) standard deviations of speed measurements within individual tracks, (B) frequency of acceleration events, and (C) magnitude of accelerations are shown for wild-type, *cat-2(n4547), goa-1(n1134)*, and *dop-3(vs106)* animals on a bacterial lawn. Tracks were first grouped by average speed. Error bars represent SEM. The average (D) standard deviations of speed measurements within individual tracks, (E) frequency of acceleration events, and (F) magnitude of accelerations are shown for dopamine pre-treated wild-type, *cat-2(n4547), goa-1(n1134)*, and *dop-3(vs106)* animals on a bacterial lawn. Tracks were first grouped by average speed. Error bars represent SEM. (wild type, n = 180 no dopamine, 189 dopamine pretreated animal tracks, *cat-2(n4547)*, n = 202, 183, *dop-3(vs106)*, n = 220, 226, *goa-1(n1134)*, n = 227, 213)

Figure 4.4

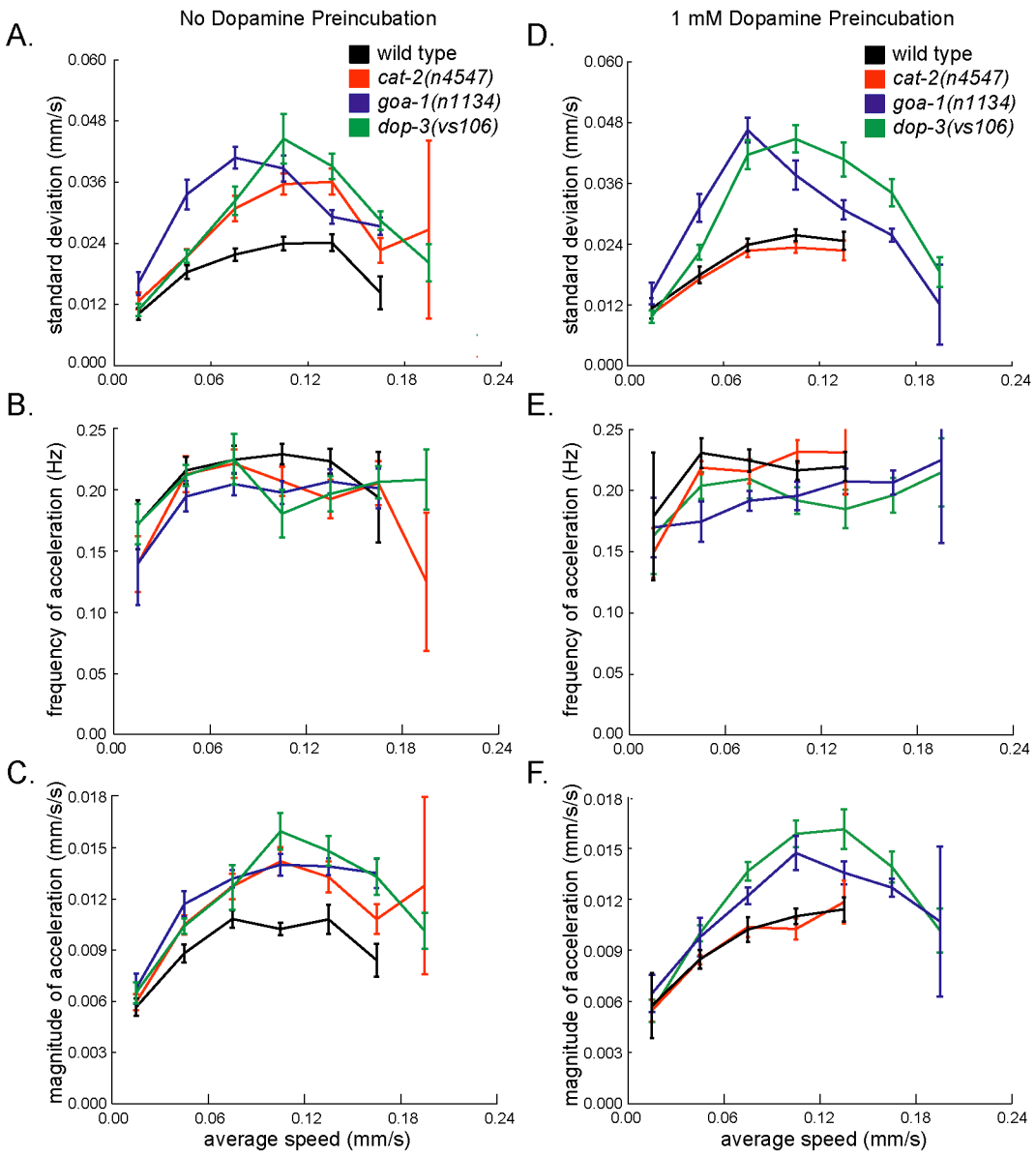

A.
No Dopamine Preincubation
D.
1 mM Dopamine Preincubation
wild type
cat-2(n4547)
goa-1(n1134)
dop-3(vs106)
standard deviation (mm/s)
B.
E.
frequency of acceleration (Hz)
C.
F.
magnitude of acceleration (mm/s/s)
average speed (mm/s)

Figure 4.5

The locomotion defects of *cat-2* mutants are suppressed by *tdc-1* mutation that blocks tyramine and octopamine synthesis. Frequency distributions of instantaneous speeds of (A) *tdc-1(n3420)* and (B) *cat-2(n4547) tdc-1(n3420)* mutants in the off-bacteria, well-fed on bacteria, and food-deprived on bacteria conditions. Wild-type frequency distribution outlines are overlaid in A and B for comparison. (C) Average speeds of wild-type and mutant animals tested in each condition. Error bars represent SEM. Asterisks indicate conditions in which the locomotion of *tbh-1* and *tdc-1* mutants are significantly different from the wild type. ($p < *.005, **.001$) The average (D) standard deviations of speed measurements within individual tracks, (E) frequency of acceleration events, and (F) magnitude of accelerations are shown for wild-type, *cat-2(n4547)*, *tdc-1(n3420)*, and *cat-2(n4547) tdc-1(n3420)* animals on a bacterial lawn. Tracks were first grouped by average speed. Error bars represent SEM. (A: *tdc-1(n3420)*, n = 173 no bacteria, 133 well-fed, 110 food-deprived tracks; B: *cat-2(n4547) tdc-1(n3420)*, n = 189, 134, 103; C: wild type, n = 1510, 1174, 967, *tbh-1(n3722)*, n = 154, 138, 118, *tdc-1(n3420)*, n = 173, 133, 110, *cat-2(n4547)*, n = 216, 160, 139, *cat-2(n4547) tdc-1(n3420)*, n = 189, 134, 103; D-F: wild type, n = 255, *cat-2(n4547)*, n = 240, *tdc-1(n3420)*, n = 243, *cat-2(n4547) tdc-1(n3420)*, n = 237)

Figure 4.5

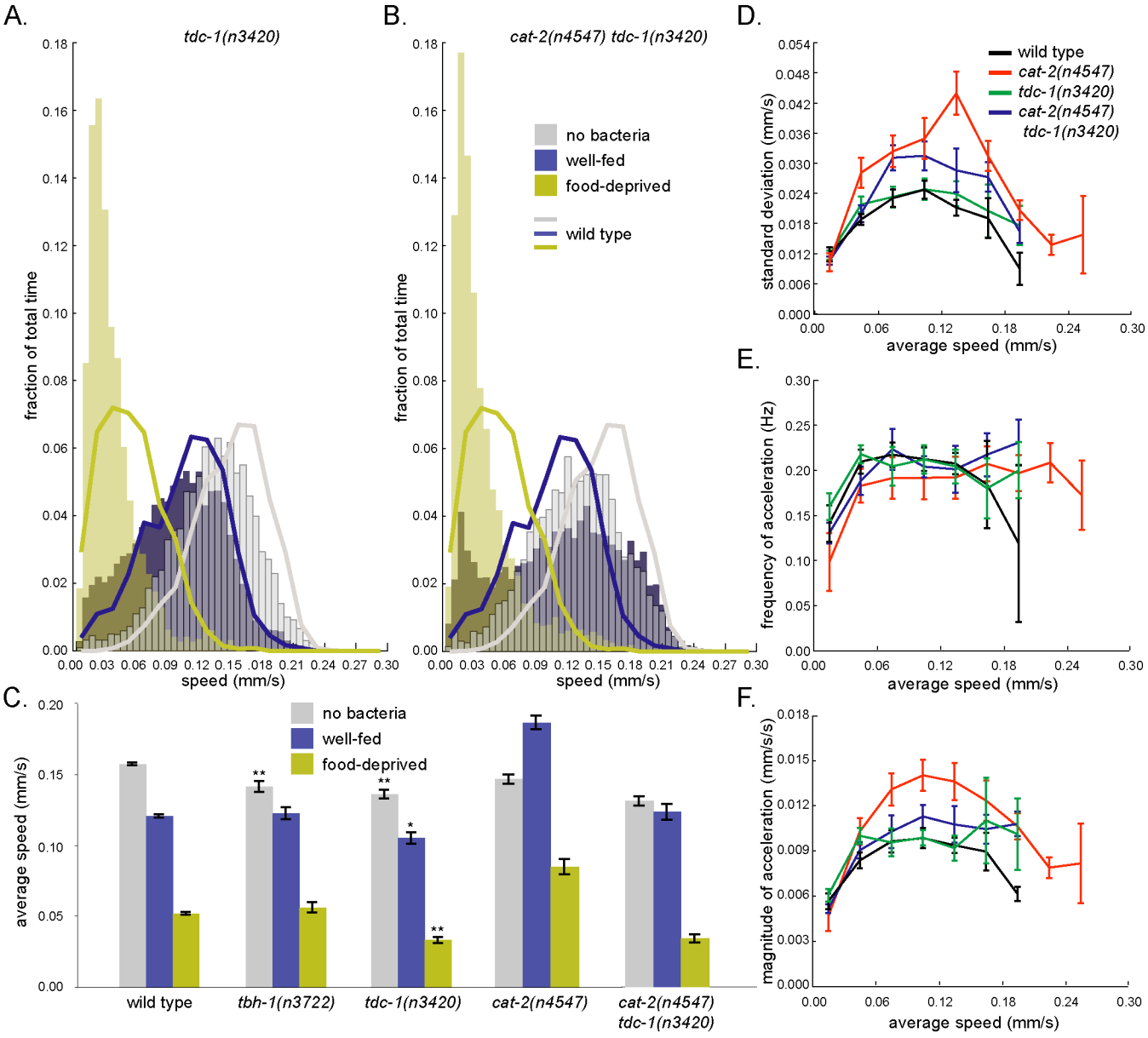

A.
tdc-1(n3420)
fraction of total time
speed (mm/s)
B.
cat-2(n4547) tdc-1(n3420)
no bacteria
well-fed
food-deprived
wild type
fraction of total time
speed (mm/s)
D.
wild type
cat-2(n4547)
tdc-1(n3420)
cat-2(n4547)
tdc-1(n3420)
standard deviation (mm/s)
average speed (mm/s)
E.
frequency of acceleration (Hz)
average speed (mm/s)
C.
no bacteria
well-fed
food-deprived
average speed (mm/s)
wild type
tbh-1(n3722)
tdc-1(n3420)
cat-2(n4547)
cat-2(n4547)
tdc-1(n3420)
F.
magnitude of acceleration (mm/s/s)
average speed (mm/s)

Figure 4.6

Model: Dopamine plays a dual role in suppressing motor activity in response to a bacterial food source and setting and maintaining a constant rate of locomotion in *C. elegans*. *C. elegans* detects activity-stimulating and activity-suppressing cues from the environment. These cues set the activity level of the animal. The speed of the animal is detected and compared to the activity setpoint, and adjustments to speed are made. If the speed of the animal is less than the activity setpoint, acceleration is initiated. If the speed of the animal is greater than the activity setpoint, deceleration is initiated. Dopamine has two distinct roles in this process. Dopamine (1) acts to reduce the activity setpoint of the animal in response to a bacterial lawn and (2) is required to accurately integrate information about the current speed of the animal, which is in turn required to set and adjust locomotion rate appropriately. Tyramine alone, or together with octopamine increases *C. elegans* response to locomotion stimulating cues and acts downstream of dopamine signaling in both processes.

Figure 4.6

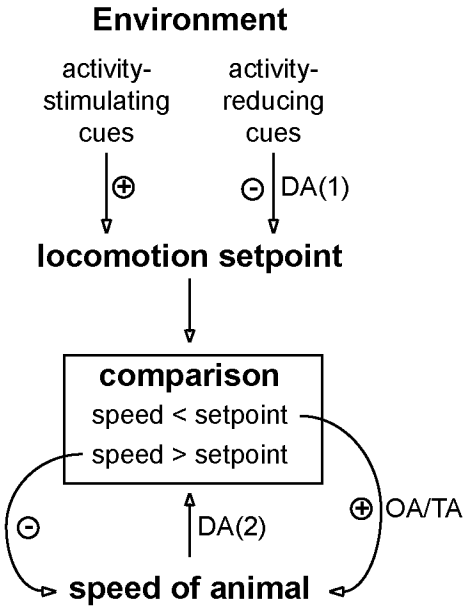
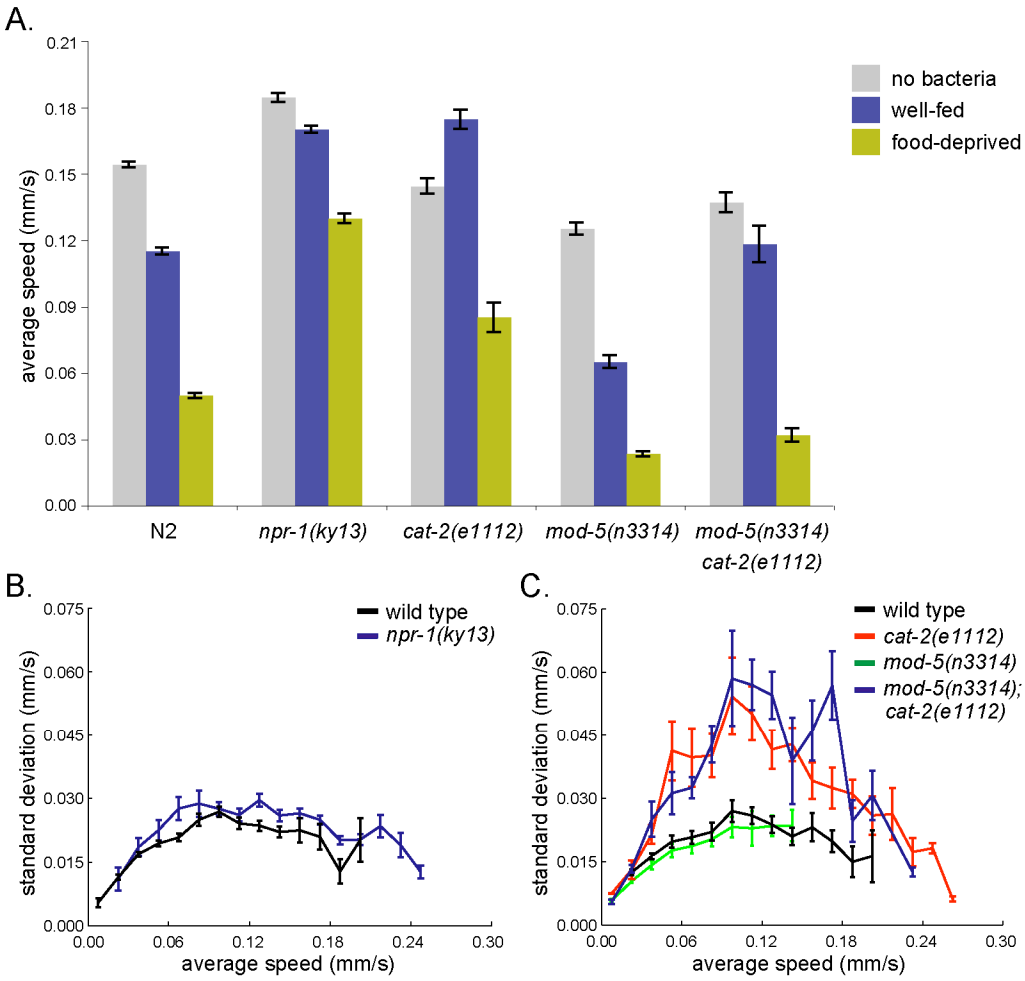

Environment
activity-stimulating cues
activity-reducing cues
DA(1)
locomotion setpoint
comparison
speed < setpoint
speed > setpoint
DA(2)
OA/TA
speed of animal

Figure 4.7

Mutations in *npr-1* and *mod-5* result in hyperactive and sluggish locomotion on bacteria, respectively, but these mutants exhibit normal variability of locomotion rate. Mutation of *mod-5* reduces the increased activity of *cat-2* mutants on a bacterial food source but does not suppress the increased fluctuations in individual locomotion rates of *cat-2* mutants. (A) Average speeds of wild-type and mutant animals tested in each condition. Error bars represent SEM. The average standard deviations of speed measurements within individual tracks are shown for (B) wild-type and *npr-1(ky13)* and (C) wild-type, *cat-2(e1112)*, *mod-5(n3314)*, and *mod-5(n3314) cat-2(e1112)* animals on a bacterial lawn. Tracks were first grouped by average speed. Error bars represent SEM. (A: wild type, n = 877 no bacteria, 678 well-fed, 532 food-deprived tracks, *npr-1(ky13)*, n = 475, 575, 412, *cat-2(e1112)*, n = 171, 123, 96, *mod-5(n3314)*, n = 226, 174, 153, *mod-5(n3314) cat-2(e1112)*, n = 117, 83, 51; B: wild type, n = 712 tracks, *npr-1(ky13)*, n = 987; C: wild type, n = 1210 tracks, *cat-2(e1112)*, n = 218, *mod-5(n3314)*, n = 327, *mod-5(n3314) cat-2(e1112)*, n = 134)

Figure 4.7


A.
average speed (mm/s)
0.21
0.18
0.15
0.12
0.09
0.06
0.03
0.00
no bacteria
well-fed
food-deprived
N2
npr-1(ky13)
cat-2(e1112)
mod-5(n3314)
mod-5(n3314) cat-2(e1112)
B.
standard deviation (mm/s)
0.075
0.060
0.045
0.030
0.015
0.000
wild type
npr-1(ky13)
0.00
0.06
0.12
0.18
0.24
0.30
average speed (mm/s)
C.
standard deviation (mm/s)
wild type
cat-2(e1112)
mod-5(n3314)
mod-5(n3314); cat-2(e1112)
average speed (mm/s)

CHAPTER 5

The competing effects of mechanical agitation and food on *C. elegans* locomotion are modulated by food-deprivation in the Mod Assay

Summary

The Mod Assay was developed to study how the behavior of *C. elegans* changes in response to food-deprivation. In this assay the locomotion rates of well-fed and food-deprived animals are measured on a bacterial food source. Upon entering a bacterial lawn, well-fed animals exhibit a small basal slowing response, while food-deprived animals exhibit a large enhanced slowing response. We created a video based locomotion tracking system that has allowed us to study the kinetics of how food-deprivation modulates locomotion and the persistence of each slowing response. Using this system we find that the onset of food-deprived behavior occurs after as little as 5 minutes of food-deprivation and corresponds with the initial absence of bacteria in the gut of the animal. As animals become food deprived, their behavior rapidly moves from basal to enhanced slowing in a rapid graded fashion. We also find that the basal slowing response reflects a transient state of elevated locomotion that is induced upon transferring well-fed animals to assay plates containing a fresh bacterial lawn. In this context, acute food deprivation appears to suppress this mechanical agitation induced increase in locomotion rate. These findings redefine the biological context within which we now think about this behavior.

Introduction

The Mod Assay was designed by previous graduate student Beth Sawin to study how the experience of being food deprived affects the locomotory behavior of *C. elegans*. Using this assay, she found that upon entering a bacterial lawn, well-fed animals exhibit a 20% reduction in their locomotion rate while food-deprived animals exhibit a 70% reduction in their locomotion rate. Food deprivation had little effect on the locomotion rate of animals in the absence of a bacterial lawn (SAWIN *et al.* 2000). The well-fed and food-deprived locomotion rates of animals on bacteria were named the basal and enhanced slowing responses, respectively.

Food-deprived animals were thought to slow more than well-fed animals upon entering a bacterial food source to maximize the time they spend in an energy rich environment. Originally, the enhanced slowing response was hypothesized to represent an aspect of learning and memory in which the previous experience of being food-deprived was stored in the neurons of the animal and used to modulate future activity. One mechanism proposed was that food-deprivation might sensitize or re-sensitize *C. elegans* to a bacterial food source (SAWIN 1996). Alternatively, the enhanced slowing response might be primarily due to changes in the gut of the animal. In the context of hunger and satiety signaling, the current metabolic state of the animal might modulate responses to a bacterial food source.

To better understand the nature of what we were studying in the Mod Assay, we decided to study the kinetics of how food-deprivation causes enhanced slowing and the duration of each locomotory response. To accomplish this task we had to overcome the challenges posed by the original Mod Assay, which included a relatively small sample size and a high degree of variability in data collected. In the original Mod Assay, the locomotion of each individual animal was determined by counting the number of body bends an animal made over a 20 second interval. Scoring locomotion by this method was time intensive and varied between investigators.

In collaboration with Damon Clark in the laboratory of Aravi Samuel in the Harvard Physics Department, we created an automated locomotion tracking system that increased the rate at which data could be collected and removed the human quantification of locomotion. After completion we found that our tracking system had the added advantage of increased temporal and spatial resolution, removal of environmental variables that affect our assay, and new types of data analysis (Appendix A). Using this system we have studied the kinetics of how food-deprivation modulates locomotion, the persistence of each slowing response, and variables that affect locomotion in the Mod Assay. These experiments have identified critical aspects of our assay that change the way in which we now think about this behavior.

Results

Our automated tracking system improves data collection and is consistent with manual scoring methods

Our automated tracking system determines the position of every animal on the assay plate at one-second intervals and assembles locomotion data of each individual worm into separate tracks, which represent the continuous speed recordings of each animal over the assay period. Using our new system we obtain average velocities for wild-type animals that are comparable to those obtained by manually counting the body bends of extremely large numbers of animals (SAWIN *et al.* 2000). Using our tracking system we found that *C. elegans* moves at an average rate of 0.16 mm/second on solid agar media in the absence of a bacterial lawn. On a bacterial lawn, animals exhibit a 24% reduction in their rate of locomotion in the well-fed condition and 68% reduction in the food-deprived condition compared to their locomotion off of food (Figure 5.1A). Manually scoring locomotion by counting the number of body bends an animals makes over a 20 second interval, we find that on a bacterial lawn, animals exhibit a 21% reduction in their locomotion rate in the well-fed condition and 70% in the food-deprived condition (Figure 5.1B).

Our automated tracking system also allows us to look at the amount of time animals move at different speeds. The instantaneous speed recordings of a population of animals can be represented by histograms. These frequency distributions can be overlaid to compare how populations of animals move under different experimental conditions (Figure 5.1C).

Thirty minutes of food-deprivation induces a rapid graded shift from basal slowing to enhanced slowing

Previous studies suggested that enhanced and basal slowing were completely different responses with respect to the mechanism by which *C. elegans* detected a bacterial lawn and the neurotransmitters each response depended on to slow the animal. The basal slowing response was reported to rely on the dopaminergic and

mechanosensory function of the ADE, PDE, and CEP neurons (SAWIN *et al.* 2000) while the enhanced slowing response was reported to rely on serotonin signaling (RANGANATHAN *et al.* 2000; SAWIN *et al.* 2000). We therefore were interested in determining whether or not animals transitioned between basal and enhanced slowing in a binary fashion or of they were able to exhibit intermediate locomotory activity.

To study the kinetics of how food-deprivation affects the modulation of locomotion we examined populations of animals that have been food-deprived for different amounts times. At food-deprivation times between 5 and 30 minutes, bi-modal frequency distributions of speed measurements suggested that two stable states of locomotory behavior exist (Figure 5.2A). Since our automated tracking system maintains the integrity of each individual track we then sub-divided the tracks of each group into fast and slow sub-groups, by a speed value between the mean basal and enhanced slowing speeds. Doing this we were able to see a shift of animals from fast to slow groups over the 30-minute food-deprivation period (Figure 5.2B).

We next studied the locomotion of transitioning animals for evidence of bi-modality in their instantaneous speed recordings. Transitioning animals are those that exhibit intermediate average locomotion rates after periods of food deprivation when many animals are beginning to exhibit enhanced slowing. We hypothesized that if animals switched their mode of locomotion in a relatively binary fashion, transitioning animals would have bimodal distributions of instantaneous locomotion rates. Dividing the group of animals that were food-deprived for 5 minutes into fast, medium, and slow sub-populations, we replotted the locomotion histograms of each of these sub-populations. Looking at animals with intermediate average velocities in this transitioning population, we find that their locomotion profile is normally distributed (Figure 5.2C). Furthermore the frequency distributions of speed measurements of individual animals from this group do not appear bimodal (data not shown). These data suggest that food-deprivation induces a rapid graded transition from fast to slow locomotory responses.

Interestingly, animals begin to exhibit greater slowing after only 5 minutes of food deprivation, which corresponds to the time it takes for animals grown on GFP labeled bacteria to clear bacteria from their gut (Figure 5.2D). These data are consistent

with a model in which locomotion is directly modulated by the detection of ingested bacteria in the gut of the animal.

The process of transferring animals to assay plates plays an important role in the basal and enhanced slowing

In the Mod Assay, scoring of locomotion began 5 minutes after moving animals to assay plates and lasted for 2 minutes. To examine the duration of each locomotory response, we recorded the locomotion of animals starting at time points beyond 5 minutes after transfer. Surprisingly we found that after 10 minutes, well-fed animals quickly reduce their rate of locomotion dramatically to levels that more closely resemble the locomotion of food-deprived animals in the enhanced slowing response (Figure 5.3B). We also find that animals not transferred to assay plates and well-fed animals 2 hours post-transfer exhibit similarly reduced locomotion rates on bacteria (Figure 5.3B,D). Since well-fed animals exhibit greatly suppressed locomotion except immediately after being transferred to assay plates, we conclude that the basal slowing response reflects a transient state of elevated locomotion induced by transferring animals to assay plates (Figure 5.3B).

In the off-food and food-deprived on bacteria conditions, the locomotion of animals is relatively stable at time points beyond 5 minutes after transfer (Figure 5.3 A,C). After acute food deprivation, animals exhibit only a very minor transient increase in locomotion rate on a bacterial lawn under standard assay conditions (Figure 5.3C). Since the locomotion of food-deprived animals is similar to the locomotion of untransferred well-fed animals, it appears that acute food deprivation suppresses the transfer-dependent stimulation of locomotion exhibited by well-fed animals.

Food deprivation is the major factor that contributes to greater slowing of animals in the Mod Assay

Since the process of transferring animals to assay plates dramatically affects the locomotion of animals on a bacterial food source we considered that unequal handling of

animals, and not food deprivation alone, might account for the difference between basal and enhanced slowing. The design of the Mod assay is such that we do not completely avoid the unequal handling of animals under well-fed and food-deprived conditions (SAWIN 1996). In the standard basal slowing assay, animals are transferred only once, to assay plates, while in the standard enhanced slowing assay, animals are transferred twice, once to food-deprivation plates and then again to assay plates (Figure 5.4A). The final transfer of food-deprived animals to assay plates is done in liquid and does not include transfer by a wire pick.

Previous investigations have shown that *C. elegans* can habituate to repeated mechanical stimuli (RANKIN *et al.* 1990), so we considered that animals transferred twice in the enhanced slowing condition might slow more because they have become habituated to the locomotion stimulating effects of the transfer process. We find that mock food-deprived animals that are first transferred to plates with a bacterial food source for 30 minutes then transferred to assay plates might exhibit some habituation to picking; however, this small difference does not account for the large difference between basal and enhanced slowing (Figure 5.4 A,B).

We also considered that transferring food-deprived animals in liquid rather than by picking might also affect locomotion. Specifically, liquid transfer might not stimulate locomotion as much as transferring animals by wire pick, thus contributing to reduced locomotion in the enhanced slowing condition. Surprisingly, we find that transferring animals by liquid appears to stimulate locomotion slightly more than transferring animals by picking (Figure 5.4B). Together our findings show that unequal handling of animals does not cause enhanced slowing and that food deprivation is indeed the main variable causing enhanced slowing.

Well-fed animals transferred to plates with old bacterial lawns do not exhibit transfer-dependent stimulation of locomotion

Transferring well-fed animals back to culture plates rather than to assay plates results in locomotion similar to untransferred animals (Figure 5.4B). Assay plates differ from culture plates in that they contain a newly grown bacterial lawn while culture plates

are typically over one week old. Thus both transferring animals to assay plates and the presence of a fresh bacterial lawn are both factors in stimulating *C. elegans* locomotion. One hypothesis is that actively dividing bacteria may produce factors that are harmful to *C. elegans* eliciting an increased locomotory response (GARIGAN *et al.* 2002; ANYANFUL *et al.* 2005). A second hypothesis is that an older thicker bacterial lawn inhibits *C. elegans* locomotion to a greater extent to a thinner bacterial lawn.

Discussion

Our findings change the biological context in which we think about the Mod Assay

The results of our investigation found that well-fed undisturbed animals exhibit greatly suppressed locomotion on bacteria and that transferring well-fed animals to assay plates stimulates locomotion. We also found that acute food deprivation appears to suppress the transfer-dependent stimulation of *C. elegans* locomotion. Thus, transferring animals to assay plates is critical to generate the different locomotory behaviors of well-fed and food-deprived worms in the Mod Assay.

We hypothesize that mechanical agitation is the important component of transferring animals to assay plates that stimulates the animal's locomotion. Both mechanical stimulation and the process of transferring animals transiently stimulate locomotion and inhibit egg-laying on bacteria (SAWIN 1996) (Niels Ringstad, personal communication). As organisms increase in complexity, they must develop strategies to integrate salient stimuli depending on the changing needs of the animal. We hypothesize that in the well-fed state, mechanical agitation has a greater effect on *C. elegans* locomotion than the bacterial cues that suppress *C. elegans* locomotion. In the food-deprived state, bacterial cues have a greater effect than the mechanical agitation that stimulates *C. elegans* locomotion. Thus, in the Mod Assay, the competing effects of agitation and bacteria on the locomotion of animals are modulated by acute food-deprivation.

The kinetics of food-deprivation impacts how we think about the different models of how food-deprivation leads to enhanced slowing

C. elegans can exhibit sensory adaptation to soluble chemicals, which elicit weaker behavioral responses after animals have been exposed to chemicals for an extended period of time (WARD 1973; WEN *et al.* 1997). In our study of sensory mutants, we see that soluble components of the bacterial lawn are likely responsible for reducing the locomotion of *C. elegans* (Chapter 2). We considered that that after extended exposure to a bacterial lawn, well-fed animals might respond less to the locomotion suppressing effects of a bacterial food source due to sensory adaptation. This adapted response can inhibit the locomotion of undisturbed well-fed animals, but is easily overridden by physically transferring animals to assay plates in the well-fed state. Acute food-deprivation re-sensitizes animals to the bacterial food source thus strengthening the locomotion inhibiting effect of a bacterial lawn on the animal. A major challenge to this model is whether or not re-sensitization can occur on the 5-minute timescale in which animals begin to exhibit enhanced slowing.

An alternative hypothesis is that hunger and satiety signaling pathways modulate the locomotory activity of the animal. Our data show that the onset of enhanced slowing correlates with the time in which bacteria is cleared from the gut of the animal. In humans and other vertebrates satiety signals like cholecystokinin originate from the stomach and intestinal lumen and signal meal termination (SCHWARTZ *et al.* 2000). In *C. elegans*, satiety signals might reduce the effect of a bacterial food source on the locomotion of animals thereby allowing other stimuli, like mechanical agitation, to modulate locomotion.

Future Directions

Our further characterization of the behavior of animals in the Mod Assay reshapes our thinking about the biological context of animals behaving in this assay but does not change the initial assertion that food-deprivation modulates the animals behavior.

Information about the well-fed or food-deprived state of the animal must be stored to modulate the animals response to agitation and food.

Sensory adaptation and metabolic signaling might both contribute to the enhanced slowing response in *C. elegans*. Studying the rate at which *C. elegans* becomes re-sensitized to various chemical cues might shed more light into whether or not food-deprivation can result in re-sensitization of animals on such a short time scale. Additionally studying the onset of the enhanced slowing response in animals that have a reduced ability to clear bacteria from their guts may shed some light onto whether hunger and satiety-like signaling modulates locomotion.

Additional experiments might also be done to study the effects of mechanical agitation on the locomotion of well-fed and food-deprived animals. These experiments might distinguish between a mechanism by which food-deprivation directly suppresses nociceptive or stress signaling pathways vs. re-sensitizing the animal to the locomotion inhibiting effects of a bacterial food source.

Methods

Cultivation and Strains

Caenorhabditis elegans were cultivated on NGM agar at 20°C as described by Brenner (1974), except that worms were grown on *E. coli* strain HB101 as a food source. The wild-type *C. elegans* strain used was N2.

Locomotion Assay

Assays were conducted according to Sawin et al. (2000) with the following modifications. Assay plates containing no bacteria or with a ring shaped bacterial lawn were examined, and only those with minimal surface imperfections and a textured non-glassy appearing bacterial lawn were used for locomotion assays. Assays were performed in a temperature controlled room at 22°C. The locomotion of 20-25 animals

was recorded in each assay. Recording of locomotion was scored by an automated locomotion tracking system and began 5 min after animals were transferred to assay plates and lasted for 2 minutes, unless otherwise noted.

We used a Sony XCD X710 firewire camera at 1024x768 resolution to capture high contrast dark field images of our assay plates illuminated from the side by a ring of LEDs. Images were acquired at 1 Hz. The centers of mass (centroids) of all worms were found and individual position recordings were assembled into tracks from centroid positions using the methods outlined by Clark et al. (2007). Animal speeds were calculated by dividing the path distance an animal traveled by the time it took to travel that path. Each speed recording was calculated by averaging 5 speed measurements observed during a 5 sec sliding window to minimize the noise in centroid displacements. Such instantaneous speed measurements varied from 0 to roughly 0.3 mm per sec.

Fluorescent Bacteria Feeding Assay

HB101 *E. coli* strains expressing bacterial optimized GFP (GFP_{UV}) were grown in LB containing 7.5 ug/mL ampicillin overnight. Standard 5 cm culture plates containing approximately 10 ml NGM agar were seeded with this culture and grown overnight into a bacterial lawn. Plates were allowed to cool to room temperature before *C. elegans* were placed on plates. L4 stage animals were picked to plates containing GFP expressing bacteria 18-24 hours before assay time. To begin the assay, animals were picked into wells of S Basal, washed free of bacteria, and placed on food-deprivation plates lacking bacteria. Animals were food deprived for 0, 5, 10, 15, 20, 25, and 30-minute periods of time. Food deprivation was stopped by passing the surface of agar plates over an open flame. This treatment does not affect GFP expression. Food deprived animals were then scored for the presence or absence of GFP signal in various internal structures of the animal using a Olympus SZX12 dissecting microscope, GFP 470 filter set, and a Leica EBQ 100 fluorescence light source.

Data Analysis

The average speed of a population was calculated to be the mean of all instantaneous speeds calculated for all animals tested. Standard error of the mean was calculated by taking the standard deviation of all speed measurements and dividing by the square root of the total number of individual tracks for all animals pooled under each condition tested. T values were determined using a student's t-test for comparing the means of two samples of unequal size. Significance was determined by finding corresponding P-values on a two-tailed student's t-distribution using our calculated t values and degrees of freedom. Locomotion assay data for each figure was collected on multiple days and pooled. Data for all conditions presented in each figure were collected on identical days.

Acknowledgments

I would like to thank my collaborators Damon Clark and Aravi Samuel who provided the expertise and inspiration to construct an automated locomotion tracking system that revolutionized our studies of *C. elegans* locomotion and made the discoveries in this chapter possible. I would also like to thank Brendan Galvin and Shunji Nakano for critical reading of this chapter and helpful comments.

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Figures

Figure 5.1

Observations of wild-type *C. elegans* locomotion obtained using an automated locomotion tracking system. (A) Average speeds of animals tested in each condition as measured by the automated tracking system. Error bars represent SEM. (B) Average speeds of animals as measured by manually counting body bends over 20 sec intervals. Error bars represent SEM. (C) Frequency distributions of instantaneous speed recordings of animals tested in the off-bacteria, well-fed on bacteria, and food-deprived on bacteria conditions. Each bin is 0.006 mm/sec wide. (A.C: n = 1510 off-food, 1174 well-fed, 967 food-deprived animal tracks, mean track length was 53 sec in the automated assay; B: n = 528 off-food, 508 well-fed, 642 food-deprived animals assayed in the manual assay).

Figure 5.1

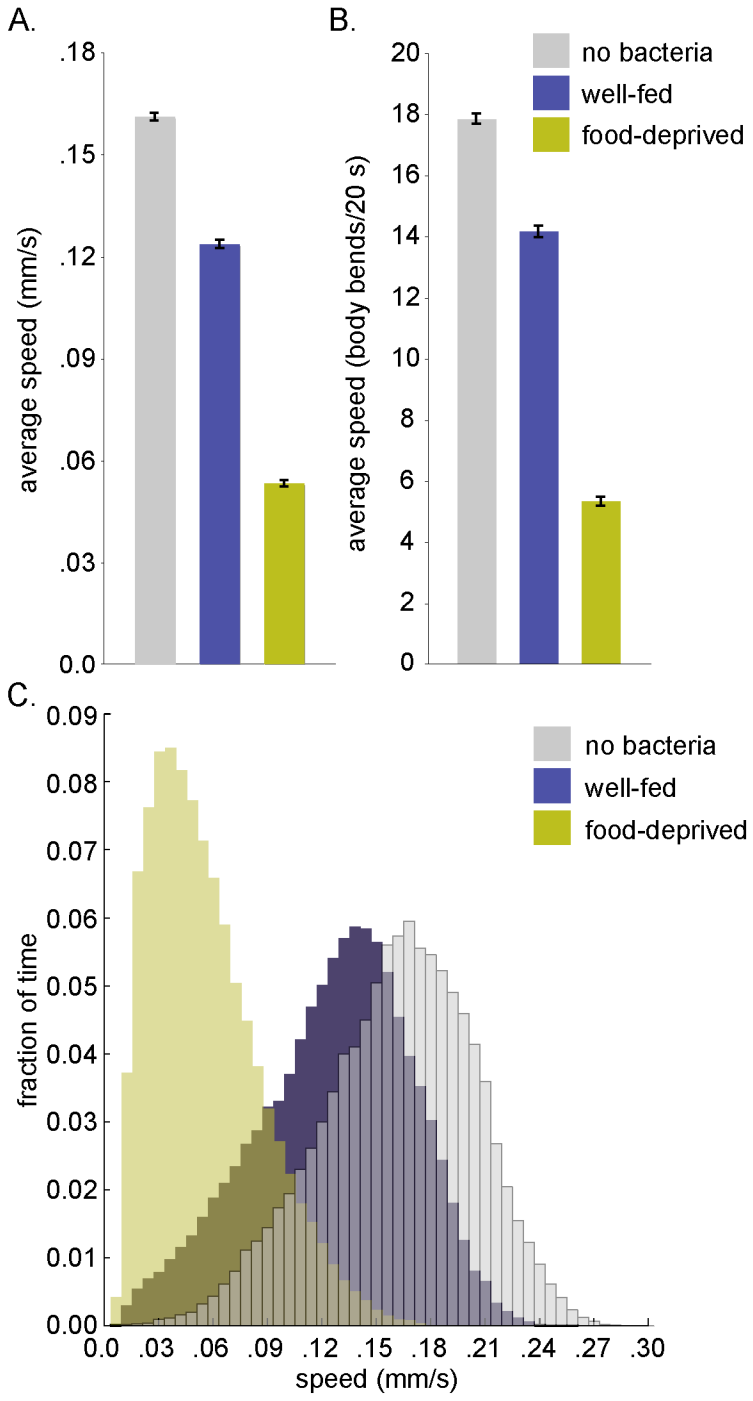

A.
B.
C.
average speed (mm/s)
average speed (body bends/20 s)
fraction of time
speed (mm/s)
no bacteria
well-fed
food-deprived
0.0
.03
.06
.09
.12
.15
.18
0
2
4
6
8
10
12
14
16
18
20
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
.21
.24
.27
.30

Figure 5.2

Animals quickly transition from elevated to reduced rates of locomotion on a bacterial food source as bacteria are cleared from the gut of the animal. (A) Frequency distribution outlines of speed measurements for wild-type animals that have been food-deprived for various amounts of time. (B) Wild-type animals were food-deprived for various amounts of time, each group was then divided into fast and slow groups based on average track velocity and graphed in paired frequency distribution outlines. (C) Animals food-deprived for 5 minutes were first divided into fast, medium, and slow groups by average track velocity and graphed as frequency distribution outlines. Simplified frequency distribution traces are plotted at 0.015 mm/second intervals. (D) As animals become food-deprived, fluorescent signals from ingested GFP labeled bacteria become diminished from the internal structures of the animal.

Figure 5.2

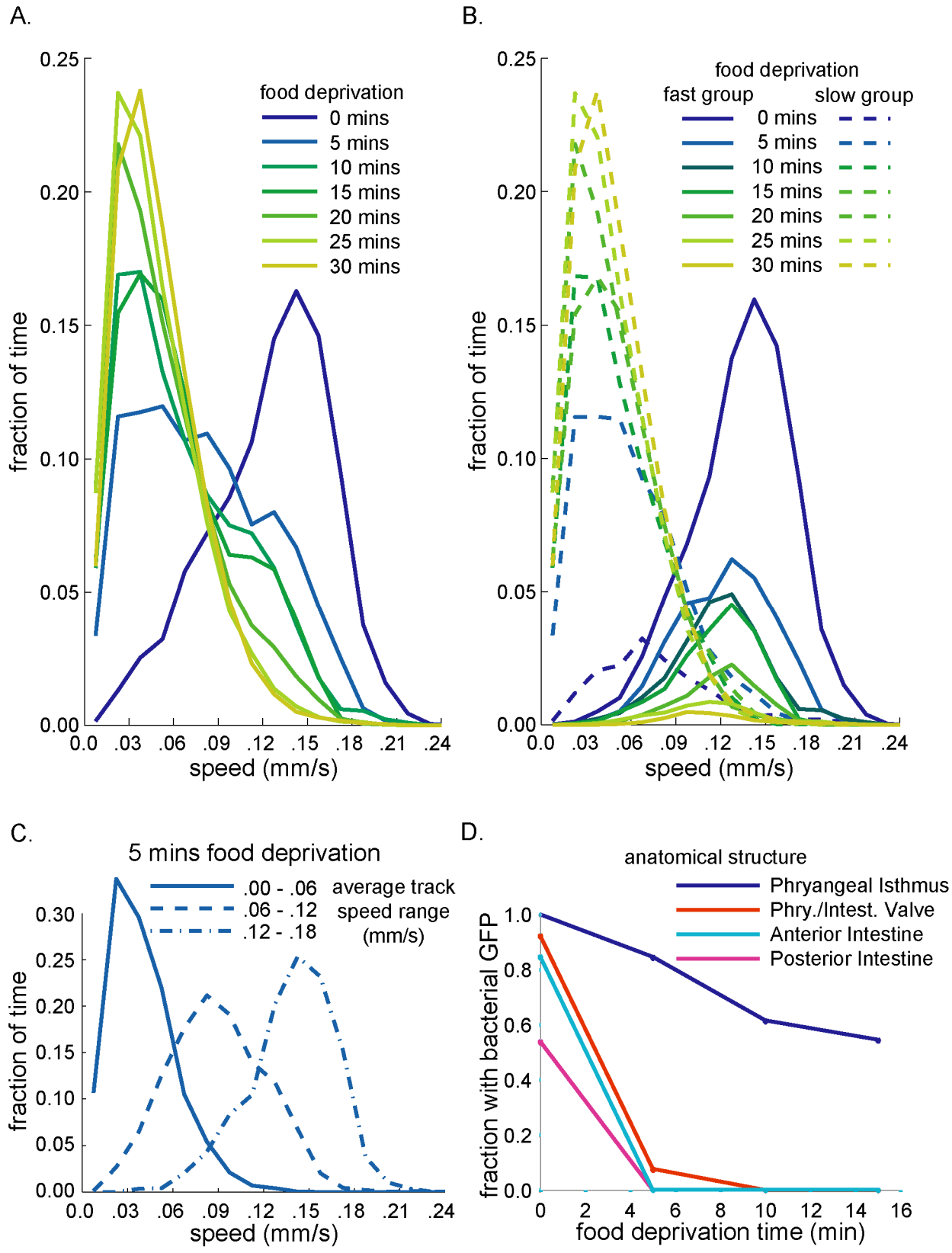

A.
B.
fraction of time
speed (mm/s)
food deprivation
0 mins
5 mins
10 mins
15 mins
20 mins
25 mins
30 mins
fast group
slow group
C.
5 mins food deprivation
.00 - .06
.06 - .12
.12 - .18
average track speed range (mm/s)
D.
anatomical structure
Phryangeal Isthmus
Phry./Intest. Valve
Anterior Intestine
Posterior Intestine
fraction with bacterial GFP
food deprivation time (min)

Figure 5.3

The duration of well-fed and food-deprived locomotory behavior on bacteria reveals a transfer dependent stimulation of *C. elegans* locomotion. Locomotion histogram outlines of animals assayed at intervals (5-35 minutes) post transfer to assay plates in (A) the off food, (B) well-fed on food, and (C) food-deprived on food conditions. (D) Locomotion histogram outline of animals moved to assay plates and left covered at room temperature for approximately 4 hours before assaying locomotion. Frequency distribution outlines are plotted at 0.015 mm/second intervals.

Figure 5.3

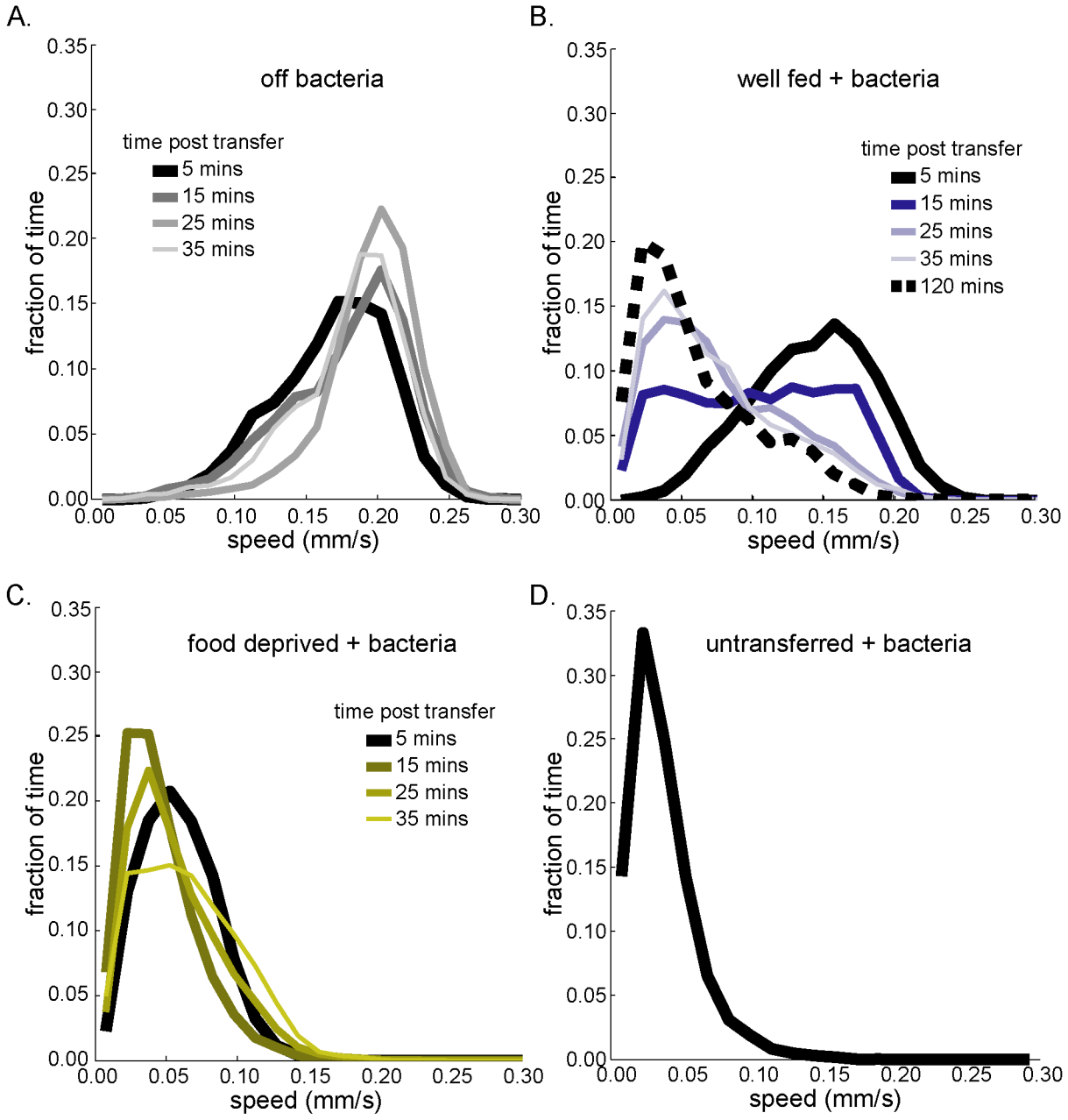
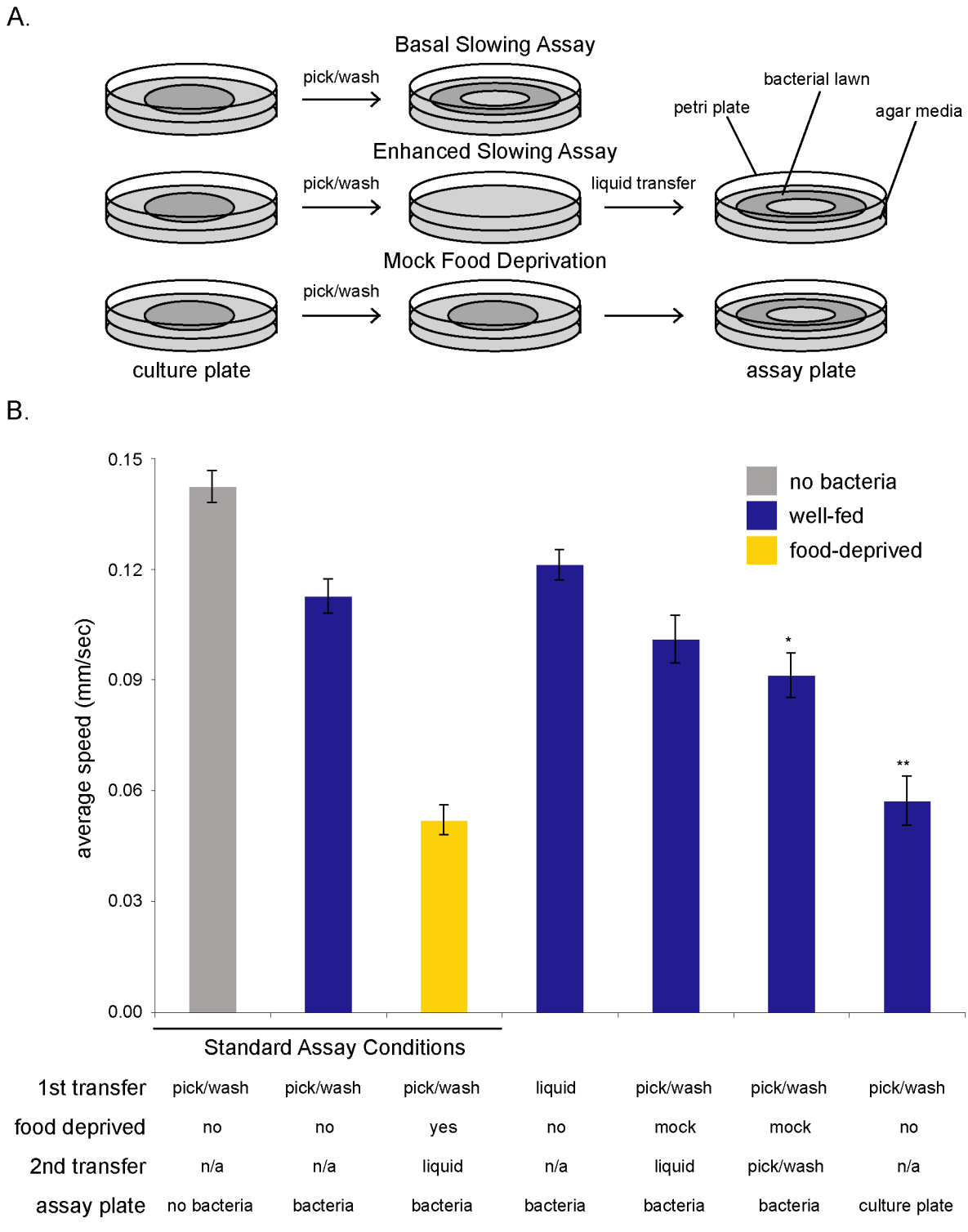

A.
off bacteria
time post transfer
5 mins
15 mins
25 mins
35 mins
fraction of time
speed (mm/s)
B.
well fed + bacteria
time post transfer
5 mins
15 mins
25 mins
35 mins
120 mins
fraction of time
speed (mm/s)
C.
food deprived + bacteria
time post transfer
5 mins
15 mins
25 mins
35 mins
fraction of time
speed (mm/s)
D.
untransferred + bacteria
fraction of time
speed (mm/s)

Figure 5.4

Unequal handling of well-fed and food-deprived animals does not account for the large difference between the basal and enhanced slowing responses. (A) Well-fed animals are picked, washed, and transferred to assay plates containing bacteria. Food-deprived animals are transferred identically to food-deprivation plates and then are transferred again to assay plates containing bacteria. Mock food-deprived animals were first transferred to plates containing bacteria and then are transferred again to assay plates containing bacteria. (B) Average velocities of animals under each condition. Error bars represent SEM. Asterisks indicate instances where the average locomotion of animals is significantly different than the average locomotion of animals assayed under canonical basal slowing conditions ($p < *.01,**.001$).

Figure 5.4


A.
Basal Slowing Assay
pick/wash
bacterial lawn
petri plate
agar media
Enhanced Slowing Assay
pick/wash
liquid transfer
Mock Food Deprivation
pick/wash
culture plate
assay plate
B.
0.15
0.12
0.09
0.06
0.03
0.00
average speed (mm/sec)
no bacteria
well-fed
food-deprived
*
**
Standard Assay Conditions
1st transfer pick/wash pick/wash pick/wash liquid pick/wash pick/wash pick/wash
food deprived no no yes no mock mock no
2nd transfer n/a n/a liquid n/a liquid pick/wash n/a
assay plate no bacteria bacteria bacteria bacteria bacteria bacteria culture plate

CHAPTER 6

Food-deprivation modulates a conserved insulin and neuropeptide Y like signaling pathway to suppress a behavioral stress response in *C. elegans*

Summary

C. elegans locomotion is greatly reduced in the presence of a bacterial food source but their locomotory activity can be stimulated by mechanical agitation. The locomotory activity of food-deprived animals however is not stimulated to the same extent by mechanical agitation. We conclude that in the well-fed condition, mechanical agitation has a greater effect on locomotion than bacteria. After food-deprivation, bacteria have a greater effect on locomotion than mechanical agitation. Thus, the competing effects of mechanical agitation and food on *C. elegans* locomotion are modulated by acute food deprivation. Presumptive metabolic and stress signaling pathways that include the insulin receptor homolog *daf-2* and the invertebrate stress hormone octopamine modulate this behavior. Genetic epistasis experiments show that these two pathways converge on the neuropeptide Y/FF-like receptor *npr-1* that appears to integrate these two signals and modulate this behavior. The ABC transporter *mrp-1* and inward rectifying potassium channel *irk-2* also regulate locomotion independently of *npr-1*.

Introduction

The acquisition of an energy source is a basic requirement of all living organisms and various strategies have evolved to facilitate this goal. Single celled bacteria can detect and chemotaxis towards a nutrient source (BERG 1975), plants coordinate their photosynthetic activity to maximize sun availability (DODD *et al.* 2005), and animals actively forage or hunt for food. At times however an organism's survival takes priority over the immediate acquisition of food. When conditions are crowded, some bacteria form stress resistant endospores to survive through unfavorable growth conditions (GROSSMAN 1995). In arid conditions plants close their stomata to prevent dehydration at the expense of carbon dioxide acquisition for photosynthesis (DODD *et al.* 2005). When imminent danger is present, animals will flee from a food-rich environment. Thus various biological drives ensure not only that an organism secures an energy source but

lives long enough to reproduce. These drives often exert opposing forces on the behaviors of the animal. Through our studies we hope to understand how these opposing drives are integrated and ultimately affect the behavior of animals.

In one context, a biological drive can be viewed as a simple homeostatic reflex that is responsive to changing internal and external environmental signals. In *The Wisdom of the Body*, Cannon defines homeostasis as the ability to maintain an internally constant environment in the face of a changing external environment. Homeostasis does not reflect a static but rather a dynamic system that is relatively constant (CANNON 1932). External and internal cues like danger or lack of food cause drives like stress or hunger that motivate behaviors that return internal stability to an organism. When opposing drives affect the same basic behavior these drives might be prioritized and we often see expression of one drive over another.

The ability to respond to stimuli and adapt to changing environmental stimuli is critical for survival. Multiple environmental cues are often concurrently integrated into adaptive responses. The relative simplicity of *Bacillus subtilis* greatly facilitated the discovery of a two-component phosphorelay system that this organism uses to integrate food and stress signals into the decision to sporulate (HOCH 1993; GROSSMAN 1995). As an organism interacts with an increasingly complex environment it must develop increasingly complex systems for integrating additional information. In humans, the integration of the large amount of information about our internal and external environmental is achieved by a complex nervous system and homeostatic mechanisms that allow an extraordinary measure of adaptability as a species.

The small free living nematode *C. elegans* is well suited for studying how competing drives like hunger and stress affect behavior. Genetic analysis of *C. elegans* is greatly facilitated by the ability to perform forward genetic screens, gene knockouts, RNA interference, transposon mutagenesis, and the ability to create transgenic strains (JOHNSEN and BAILLIE 1997). *C. elegans* respond to a wide range of stimuli (WARD 1973; HEDGECOCK and RUSSELL 1975; CULOTTI and RUSSELL 1978; CHALFIE *et al.* 1985; BARGMANN *et al.* 1993; KAPLAN and HORVITZ 1993), exhibit behavioral plasticity (WARD 1973; HEDGECOCK and RUSSELL 1975; DUSENBERY 1980; RANKIN *et al.* 1990; COLBERT and BARGMANN 1995; WEN *et al.* 1997), and have a simple yet highly

conserved nervous system (WHITE *et al.* 1986; BARGMANN 1998). *C. elegans* also exhibit developmental and behavioral responses to food and stress (HORVITZ *et al.* 1982; GOLDEN and RIDDLE 1984b; AVERY and HORVITZ 1990; RANKIN *et al.* 1990),

The early larva surveys the environment to assess whether current conditions are favorable for growth and reproduction. Under favorable conditions, the early larvae develops directly into an adult; under unfavorable conditions, the larvae can enter a stress-resistant larval state called dauer (CASSADA and RUSSELL 1975). Dauer larvae are resistant to detergents, desiccation, heat, and can live for months before exiting dauer when conditions improve (CASSADA and RUSSELL 1975). The presence of a bacterial food source inhibits dauer formation while high temperature and crowded conditions promote dauer development (GOLDEN and RIDDLE 1982; GOLDEN and RIDDLE 1984a). The balance between these factors affects the decision to enter dauer or continue immediately into developing into a reproductive adult (GOLDEN and RIDDLE 1984b).

C. elegans also exhibit behavior in which the competing effects of food and mechanical agitation modulate the locomotion of the animal. The locomotion of well-fed undisturbed animals is greatly suppressed in the presence of a bacterial food source. In the Mod Assay, transferring well-fed animals to assay plates transiently elevates locomotion in the presence of a bacterial food source (Chapter 5). Thus in the well-fed condition, mechanical agitation has a temporarily greater effect on locomotion rate than the bacterial lawn. Transferring food-deprived animal's to assay plates does not cause elevated locomotion (Chapter 5). In the food-deprived condition, the effect of bacteria has a greater effect on the locomotion of animals than mechanical agitation. Thus acute food-deprivation causes a shift in the relative response of animals to food and mechanical agitation (SAWIN *et al.* 2000) (Chapter 5).

Our investigation started with the observation that well-fed and food-deprived animals exhibit very different rates locomotion on a bacterial lawn in the Mod Assay. We began by looking for mutants that exhibited reduced locomotion on bacteria constitutively, consistent with reduced sensitivity to mechanical agitation or perception of a constitutively food-deprived state. Our genetic screen led us to investigate mutants that form constitutive dauers which might have a reduced ability to detect nutrients from a bacterial food source that inhibit dauer formation. We next looked at the role of

conserved invertebrate stress signaling molecules in this behavior. Finally we looked at mutants that exhibited altered locomotory responses in the Mod Assay consistent with increased sensitivity to mechanical agitation or the perception of a constitutively well-fed state. Using genetic epistasis analysis we placed these genes into a genetic pathway.

Results and Discussion

A genetic screen for constitutively hungry mutants

Well-fed animals move faster than food-deprived animals on a bacterial food source in the Mod Assay (SAWIN *et al.* 2000). Since the locomotion of animals on bacteria depends on whether or not they were previously food deprived, this difference in locomotion rate indicates whether an animal perceives a well-fed or food-deprived state. To identify genes that are required to perceive the well-fed state, we screened for mutants that appear constitutively food-deprived in our locomotion assay. Specifically, we isolated mutants that exhibit constitutively reduced locomotion on bacteria independent of feeding state.

We began our mutant hunt in a sensitized genetic background containing a mutation in the serotonin reuptake transporter *mod-5*. *mod-5* mutants move slowly on a bacteria food source in the well-fed condition and become paralyzed on a bacterial food source in the food-deprived condition (SAWIN 1996; RANGANATHAN *et al.* 2001) (Figure 6.1A) . In our screen, we looked for mutants that exhibit food-dependent paralysis without being food-deprived. To eliminate isolates with general locomotion defects or isolates that were food-deprived due to defects in food consumption or absorption, we selected only mutants that move well in the absence of bacteria and developed at normal rates (Appendix B).

The ABC transporter mrp-1 might have a role in transducing food signals in the modulation of locomotion and dauer formation

In this screen we isolated a mutant with a mutation in the *C. elegans* ABC transporter *mrp-1(n4167)* (multidrug resistance protein family) which encodes a member of a large family of ATP Binding Cassette (ABC) transporters (HIGGINS 1992; BROEKS *et al.* 1996). In a *mod-5* background *mrp-1* mutants become paralyzed upon entering a bacterial food source in the well-fed state (Figure 6.1B). In the absence of a *mod-5* mutation, however *mrp-1* mutants exhibit generally reduced locomotion (Figure 6.1C).

mrp-1 is similar to the human sulfonylurea receptor SUR1, which acts in the SUR1/K_{IR}6 inward rectifying potassium channel complex that is sensitive to changing ATP/ADP levels and is thought to be an important component in glucose homeostasis in many cell types (NICHOLS 2006). Mutants with mutations in the *C. elegans* inward rectifying potassium channel *irk-2*, have similarly reduced locomotion upon entering a bacterial food source (Figure 6.1C). At this point it was unclear whether or not generally reduced locomotion of *mrp-1* and *irk-2* mutants reflected a defect in satiety signaling or an unrelated inability to stimulate locomotion.

mrp-1 is expressed in a number of anatomical structures most of which are in direct contact with ingested food (BROEKS *et al.* 1996). Our *mrp-1::GFP* transcriptional reporter gene is expressed in the gland cells, pharyngeal intestinal valve, and interior intestine of the animal (Figure 6.1D). This reporter is also expressed in the vulval cells and rectal gland cells (Figure 6.1EF). Our anti-MRP-1 antibodies also stain the membranes of these structures (data not shown). Exploring metabolism related disorders in *C. elegans* we found that *mrp-1* mutants exhibit normal fat accumulation, sensitivity to cholesterol deprivation, and lifespan (data not shown). *mrp-1* mutants do however form constitutive dauers in a sensitized background as reported by Yabe et al. (2005).

Dauer development is stimulated by high temperatures and high concentrations of dauer pheromone which indicates crowded conditions, and is inhibited by a bacterial food source (GOLDEN and RIDDLE 1982; GOLDEN and RIDDLE 1984a). We considered that an animal that perceives a constitutive state of food-deprivation might have an increased rate of dauer formation because it fails to detect bacterial food-signals either externally or metabolically. Since *mrp-1* mutants exhibit locomotion characteristic of constitutively food-deprived animals in a *mod-5* background, forms constitutive dauers, and MRP-1 is

expressed in internal structures in contact with bacteria, we hypothesize that *mrp-1* might have a role in the detection of bacteria metabolites that signal the well-fed state.

The insulin receptor homlog daf-2 might act as a food-related signal in the developmental decision to form dauers and the modulation of locomotion by food-deprivation

Upon our characterization of *mrp-1* mutants, we looked for similar locomotion defects in mutants that form constitutive dauers. Experimental evidence suggests that dauer pheromone stimulates dauer formation through inhibition of a TGF-β signaling pathway (REN *et al.* 1996; SCHACKWITZ *et al.* 1996). It remains less clear how the presence of a bacterial lawn inhibits dauer formation. Mutations in genes that encode the TGF-β-like growth factor DAF-7, TGF-β/Activin-like receptor DAF-1, and SMAD protein DAF-8 cause the formation of constitutive dauers (GEORGI *et al.* 1990; REN *et al.* 1996; RIDDLE and ALBERT 1997). However, unlike *mrp-1* mutants, these dauer mutants do not exhibit reduced locomotion on bacteria (Figure 6.2A).

By contrast, mutation of the insulin-like receptor *daf-2* results in both the formation of constitutive dauers (KIMURA *et al.* 1997) and reduced locomotion off and on bacterial food source (Figure 6.2A). Like *mrp-1* mutants, *daf-2* mutants exhibit constitutive food-dependent paralysis animals in a *mod-5* background (Figure 6.2B). In addition to dauer formation, *daf-2* is involved in a number of other processes associated with metabolism in *C. elegans* including regulation of fat accumulation and lifespan (KENYON *et al.* 1993; OGG *et al.* 1997). In mammals, the peptide hormone insulin is released upon consumption of food and can inhibit food intake (WOODS *et al.* 1979; SIPOLS *et al.* 1995; SCHWARTZ *et al.* 2000). Our results suggest that signaling through the insulin receptor DAF-2 might be a common mechanism for the integration of food signals in both the modulation of locomotion and inhibition of dauer development.

The invertebrate stress hormone octopamine and/or tyramine stimulates locomotion in C. elegans

A noxious stimulus like mechanical agitation increases the locomotion rate of animals (RANKIN *et al.* 1990). To investigate the role of a known invertebrate stress hormone octopamine (ROEDER 1999) in this behavior we looked at mutants that perturb the synthesis of octopamine. Octopamine is involved in many neurological processes in including modulation of sensory functions (DAVENPORT 1984; ROEDER 1999). The *C. elegans* gene *tdc-1* encodes a tyrosine decarboxylase enzyme that is required for the synthesis of octopamine and its biologically active precursor tyramine (ALKEMA *et al.* 2005). *tdc-1* mutants have reduced locomotory activity consistent with a role for octopamine in either signaling the well-fed state or sensitizing the animal to mechanical agitation (Figure 6.2C). Given the role of octopamine as a stress hormone in many other invertebrate systems, we hypothesize that octopamine is likely involved in sensitizing the animal to mechanical agitation.

Mutation of the neuropeptide Y/FF-like receptor npr-1 causes behaviors consistent with loss of hunger and anti-nociceptive signaling

We next attempted to identify mutants that remained sensitive to mechanical agitation even after they have been food deprived. In early mapping experiments using the polymorphic wild strain CB4856, we observed that in the Mod Assay, this strain has increased activity on a bacterial food source compared to the N2 wild-type strain used in most of our experiments (Figure 6.3A). Previously, DeBono and Bargmann identified a polymorphism in the neuropeptide receptor homologue *npr-1* that causes the CB4856 strain to move faster on a bacterial food source compared to the N2 wild type and that loss-of-function mutations in the *npr-1* locus also caused the same defects (DE BONO and BARGMANN 1998).

The *C. elegans* gene *npr-1* encodes a G-protein coupled receptor that is most similar to the neuropeptide Y/FF family of receptors. NPR-1 is involved in social feeding, aerotaxis, and locomotory responses to bacteria in *C. elegans* (DE BONO and BARGMANN 1998; COATES and DE BONO 2002; DE BONO *et al.* 2002; CHEUNG *et al.* 2005). In mammals, neuropeptide Y signaling functions in energy homeostasis and stimulates food consumption in response to a deficit in energy stores thus acting as a

hunger signal (SCHWARTZ *et al.* 2000). Neuropeptide Y also appears to strongly suppress behavioral stress responses in vertebrates (HEILIG 2004). Injection of neuropeptide Y into the brains of rats has both anti-anxiety effects and can potently stimulate food intake (STANLEY *et al.* 1986; HEILIG *et al.* 1993). Neuropeptide FF modulates pain responses and both inhibits and potentiates the analgesic effects of morphine (PANULA *et al.* 1999).

We found that in the Mod Assay, *npr-1* mutants exhibit increased activity on a bacterial food source in both the well-fed and food-deprived conditions (Figure 6.3A). Conversely, overexpression of *npr-1* reduced the locomotion of animals entering a bacterial food source in the well-fed and food-deprived conditions (Figure 6.3B). Mutations of the identified *npr-1* ligands *flp-18* and *flp-21* do not affect the locomotion of mutants carrying mutations separately or together (Appendix C). Mutations in the highly similar gene *npr-2* cause a different locomotion defect in which mutants move very little both in the presence and absence of bacteria (Appendix C).

We considered that the elevated locomotion rates of *npr-1* mutants on bacteria might be caused by attenuation of signaling pathways involved in either hunger signaling or reducing responses to stressful stimuli as in mammals. A defect in hunger signaling might result in mutants that remain sensitive to mechanical stimulus because they never perceive a state of food deprivation, whereas a defect in signaling that antagonizes stress responses might result in mutants that can not down-regulate responses to mechanical stimuli. To explore these possibilities we preformed genetic epistasis experiments to determine how neuropeptide signaling through *npr-1* interacted with presumptive metabolic and stress signaling pathways.

npr-1 signaling acts downstream of presumptive metabolic and stress pathways in C. elegans

The increase in locomotion caused by a null mutation in *npr-1* was epistatic to the reduction of locomotion induced by a *daf-2* loss-of-function mutation (Figure 6.4A). These data are consistent with *npr-1* acting downstream of this insulin-like signaling pathway. Thus *daf-2* appears to act through the inhibition of neuropeptide signaling through *npr-1* in the modulation of locomotion (Figure 6.4B). *npr-1* mutations did not

suppress the dauer-constitutive phenotype of *daf-2* mutants (data not shown). In mammals, insulin signaling reduces food intake by inhibiting neuropeptide Y signaling, which stimulates food-intake (SCHWARTZ *et al.* 1992; SIPOLS *et al.* 1995; SCHWARTZ *et al.* 2000). We hypothesize that the genetic interactions we observed between insulin and neuropeptide signaling suggest the existence of a primordial food-detection system.

The increase in locomotion caused by *npr-1* mutation was also epistatic to the reduction of locomotion induced by a deletion of *tdc-1*, which is consistent with *npr-1* acting downstream of the stress hormone octopamine (Figure 6.4C). Thus octopaminergic signaling might also act through the inhibition of the *npr-1* neuropeptide-signaling pathway in the modulation of locomotion (Figure 6.4D). Neuropeptide Y is present in most noradrenergic neurons in the human sympathetic nervous system and has been shown both to potentiate the effects of this neurotransmitter post-synaptically and to inhibit release of noradrenaline (WAHLESTEDT and REIS 1993). Octopamine is the invertebrate analog of norepinephrine. Our data suggest that octopamine shares a close relationship with neuropeptide signaling in *C. elegans* as norepinephrine does in humans.

mrp-1 acts in parallel to npr-1 signaling

We tested genetic interactions between *npr-1* and *mrp-1* and observed an intermediate locomotion phenotype in *mrp-1 npr-1* double mutants (data not shown). We conclude that *mrp-1* likely acts in parallel to *npr-1*. Since *npr-1* and *daf-2* appear to act in the same pathway with respect to the modulation of locomotion, these data suggest that *mrp-1* and *daf-2* act in parallel pathways with respect to some aspects of this behavior. In pancreatic β-cells, the sulfonylurea receptor/inward rectifying potassium channel (SUR1/Kir6) complex acts as a metabolic sensor responding to levels of ADP and ATP directly affecting the release of insulin (NICHOLS 2006). Loss of the SUR-1/Kir6 channel function would cause an increase in insulin signaling. However, as reported earlier, we observed that mutations of *mrp-1*, *irk-2*, and *daf-2* causes similar locomotion defects. Thus the close relationship between the SUR1/Kir6.2 complex and insulin signaling in

humans does not appear to exist between *mrp-1*/*irk-2* and *daf-2* with respect to modulation of locomotion in *C. elegans*.

Mutations in the genes encoding the C. elegans guanyl cyclase daf-11 and HSP90 homolog daf-21 might cause constitutive enhanced slowing

In our survey to identify animals that exhibit constitutively food-deprived behavior, we looked at mutants that form constitutive dauers. We found that mutation of *daf-11* appears to cause animals to exhibit reduced locomotion, characteristic of food-deprived animals, in both the well-fed and food-deprived states while the locomotion of animals in the absence of bacteria is unaffected (Figure 6.5A). On two separate occasions we also tested the same *daf-21* mutant strain. On one occasion, these mutants exhibited reduced locomotion, characteristic of food-deprived animals (Figure 6.5C), however upon a subsequent retest these animals exhibited roughly normal rates of locomotion in our assay (data not shown). *daf-11* encodes a guanyl cyclase and *daf-21* encodes a 90 kD heat-shock protein (HSP90) (BIRNBY *et al.* 2000). In addition to forming constitutive dauers and having a modulation of locomotion defect, *daf-11* and *daf-21* mutants are also defective in the detection of a number of soluble and volatile chemicals. (VOWELS and THOMAS 1994; BIRNBY *et al.* 2000). *tax-2* and *tax-4* encode cyclic nucleotide gated channel proteins and mutations in these genes cause extensive chemosensory defects (COBURN *et al.* 1998) and similarly reduced locomotion on a bacterial food source (Figure 6.5A). Earlier we showed that *tax-2* and *tax-4* might be required to maintain sensory function that is involved in the reception of cues that stimulate the locomotion of animals on a bacterial food source (Chapter 2).

It has been reported that mutations in *tax-2* and *tax-4* suppress the hyperactivity of *npr-1* mutations and that these genes act downstream of *npr-1* (COATES and DE BONO 2002). Using our automated locomotion tracking system we found that this suppression is incomplete. The *npr-1(ky13)* mutation increased the locomotion rates of *tax-2(p671)* and *tax-4(p678)* mutants (Figure 6.5A). Similarly, *npr-1(ky13)* also increased the locomotion rates of *daf-11(m47)* mutants (Figure 6.5A). These results suggest that *daf-11*, *tax-2*, and *tax-4* act in parallel to *npr-1*. This interpretation might be complicated

by the nature of *tax-2(p671)*, which is likely not a complete loss-of-function allele (BIRNBY *et al.* 2000). Additionally, the nature of the *daf-11(m47)* lesion is undefined (BIRNBY *et al.* 2000) (Figure 6.5B). An alternative hypothesis is that these genes might act redundantly downstream of *npr-1* (BIRNBY *et al.* 2000).

Though *daf-11* and *daf-21* both have sensory defects and form constitutive dauers, mutation of the *C. elegans* HSP90 homologue *daf-21* causes much weaker chemosensory and dauer formation defects compared to those caused by *daf-11* mutation (VOWELS and THOMAS 1994; COBURN *et al.* 1998; INOUE *et al.* 2003). *daf-21* is also ubiquitously expressed unlike *daf-11*, *tax-2*, and *tax-4*, which are all expressed in chemosensory neurons (COBURN and BARGMANN 1996; KOMATSU *et al.* 1996; BIRNBY *et al.* 2000). The *daf-21(p672) npr-1(ky13)* double mutant was built twice and tested twice in epistasis tests. On the first occasion, the locomotion defect of *daf-21(p673)* mutants appeared completely epistatic to the increased locomotion of *npr-1* (Figure 6.5C). From this we might conclude that though the *p673* mutation is likely to only cause a partial loss-of-function of *daf-21* (BIRNBY *et al.* 2000), *daf-21* likely functions downstream of *npr-1* in the modulation of *C. elegans* locomotion (Figure 6.5D). Upon rebuilding the *daf-21 npr-1* double mutant, we were unable to confirm this result and are thus unsure of results involving *daf-21*. Further experiments are ongoing to determine the reasons for these ambiguous results with respect to *daf-21*.

Conclusions

We found that signaling through *daf-2* and *tdc-1* both rely on the neuropeptide receptor *npr-1* to modulate the sensitivity of *C. elegans* to a stress induced locomotory response. We propose a model in which the stress hormone octopamine and insulin-related satiety signals sensitize the animal to mechanical stimuli by suppressing *npr-1* which reduces the animals response to noxious stimuli via an anti-anxiety related capacity (Figure 6.6). We hypothesize also that *daf-11*, *tax-2*, and *tax-4* might be involved in sensory processes that detect a noxious stimuli that stimulates *C. elegans* locomotion in this behavior, which is consistent with these genes acting in a parallel but related process (Figure 6.6). The involvement of *npr-1* in both insulin-related and

octopamine signaling pathways suggests that *npr-1* might play a critical role in the integration of metabolic and stress signals that modulate *C. elegans* locomotion. In mammals, neuropeptide Y signaling appears to act centrally in energy metabolism, stress signaling, and anxiety (SCHWARTZ *et al.* 2000; HEILIG 2004). In *C. elegans*, we similarly find that the neuropeptide receptor *npr-1* appears to be involved in the organism's food and stress responses.

Methods

Cultivation and Strains

All *Caenorhabditis elegans* strains were cultivated on NGM agar at 20°C as described by Brenner (1974), except that worms were grown on the *E. coli* strain HB101 as a food source. Worms were grown in non-crowded conditions. The wild-type *C. elegans* strain used was N2. Strains used include CB1370 *daf-2(e1370),* CB1372 *daf-7(e1372),* CB1393 *daf-8(e1393),* CB3332 *che-12(e1812),* CB4856 wild type, CX4 *odr-7(ky4),* CX32 *odr-10(ky32),* CX4148 *npr-1(ky13),* DR47 *daf-11(m47),* MT890 *daf-1(m40),* MT3571 *osm-8(n1518),* MT3600 *osm-3(p802),* MT3602 *osm-9(n1603),* MT4583 *odr-1(n1930),* MT4592 *odr-2(n1939),* MT5306 *odr-3(n2150),* MT9772 *mod-5(n3314),* MT10661 *tdc-1(n3420),* MT13092 *mod-5(n3314); mrp-1(n4167),* MT13709 *mod-5(n3314); mrp-1(pk89),* MT14415 *mod-5(n3314); daf-2(e1370),* MT15610 *flp-18(n4766)*, MT15614 *tax-2(p671); npr-1(ky13),* MT15615 *tax-4(p678); npr-1(ky13),* MT15623 *npr-1(ky13) lin-15AB(n765ts); nEx1252,* MT15624 *tdc-1(n3420); npr-1(ky13),* MT15935 *irk-2(n4896),* MT16037 *lin-15AB(n765ts); nEx1252,* MT16040 *tdc-1(n3420); lin-15AB(n765ts); nEx1252,* MT16876 *daf-2(e1370); npr-1(ky13),* MT17148 *flp-21(ok889)*; *flp-18(n4766)*, MT17377 *mrp-1(pk89) npr-1(ky13), npr-1 gfp, daf-11(m47); npr-1(ky13), daf-21(p673); npr-1(ky13),* NL147 *mrp-1(pk89),* PR671 *tax-2(p671),* PR673 *daf-21(p673),* PR674 *che-1(p674),* PR678 *tax-4(p678)*, RB982 *flp-21(ok889)*, XA3702 *npr-2(ok419)*.

Modulation of Locomotion Assay

Assays were performed according to Sawin et al. (2000). Assay plates containing no bacteria or a ring-shaped bacterial lawn were examined, and only those with minimal surface imperfections and a textured non-glassy appearing bacterial lawn were used for locomotion assays. Animals were deprived of food for 30 minutes. Animals were assayed 5 min after their transfer to assay plates for 2 min.

We used two methods to score the locomotion of animals. The first was to count the number of body bends an animal makes in a 20 second period according to Sawin et al. 2000 (SAWIN *et al.* 2000). The second scoring method used a Sony XCD X710 firewire camera at 1024x768 resolution to capture high-contrast dark-field images of our assay plates illuminated by a ring of LEDs. Images were acquired at 1 Hz. The centers of mass (centroids) of all worms were found and individual position recordings were assembled into tracks from centroid positions using the methods outlined by Clark et al. (2007). Animal speeds were calculated by dividing the path distance an animal traveled by the time it took to travel that path. Speed recordings was calculated by averaging 5 speed measurements observed during a 5 sec sliding window to minimize the noise in centroid displacements. Scoring locomotion using both of these methods result in identical modulation of locomotion data collected for wild-type animals.

Data Analysis

The average speed of a population was calculated to be the mean of all speed recordings calculated for all animals tested. T values were determined using a student's t-test for comparing the means of two samples of unequal size. P-values were determined from a two-tailed student's t-distribution.

In the manual assay, to compare the average locomotion rates for mutants assayed on different days, all data was collected and pooled from multiple days and different days and plotted together. In our automated tracking assay, to compare the average locomotion rates for mutants assayed on different days, we calibrated our results using paired wild-type controls collected on every assay day. First wild-type control data from

all days were pooled to create a master wild-type dataset. A paired wild-type dataset was then created for each mutant, by pooling the wild-type control data for each day that specific mutant was assayed. To standardize data to the master control, we then multiplied the average speed of each mutant in each condition (off-food, well-fed, food-deprived) by the ratio between the master wild-type control and paired wild-type control (value Master / value Paired) for each condition. We found that this method most successfully maintained the integrity of the relationships between mutants when compared to data collected completely in parallel.

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Figures

Figure 6.1

Mutations in *mrp-1* cause constitutive food-dependent paralysis in a *mod-5* background. *mrp-1* and *irk-2* mutations alone cause generally reduced locomotion. MRP-1 is expressed in many anatomical structures that are in direct contact with ingested bacteria. (A) Average speed of wild-type and *mod-5(n3314)* mutants tested in each condition as measured manually. (B) Average speed of *mod-5(n3314)*, *mod-5(n3314); mrp-1(n4167)*, and *mod-5(n3314);mrp-1(pk89)* animals tested in the off-food and well-fed on bacteria conditions as measured manually. (C) Average speed of wild-type, *mrp-1(pk89)*, and *irk-2(n4896)* mutants tested in each condition as measured by an automated locomotion tracking system. Error bars represent SEM. (D-F) An 3.3kb *mrp-1* promoter fragment drove expression of GFP in the pharyngeal intestinal valve, anterior intestine, rectal gland cell, and vulval tissue.

Figure 6.1

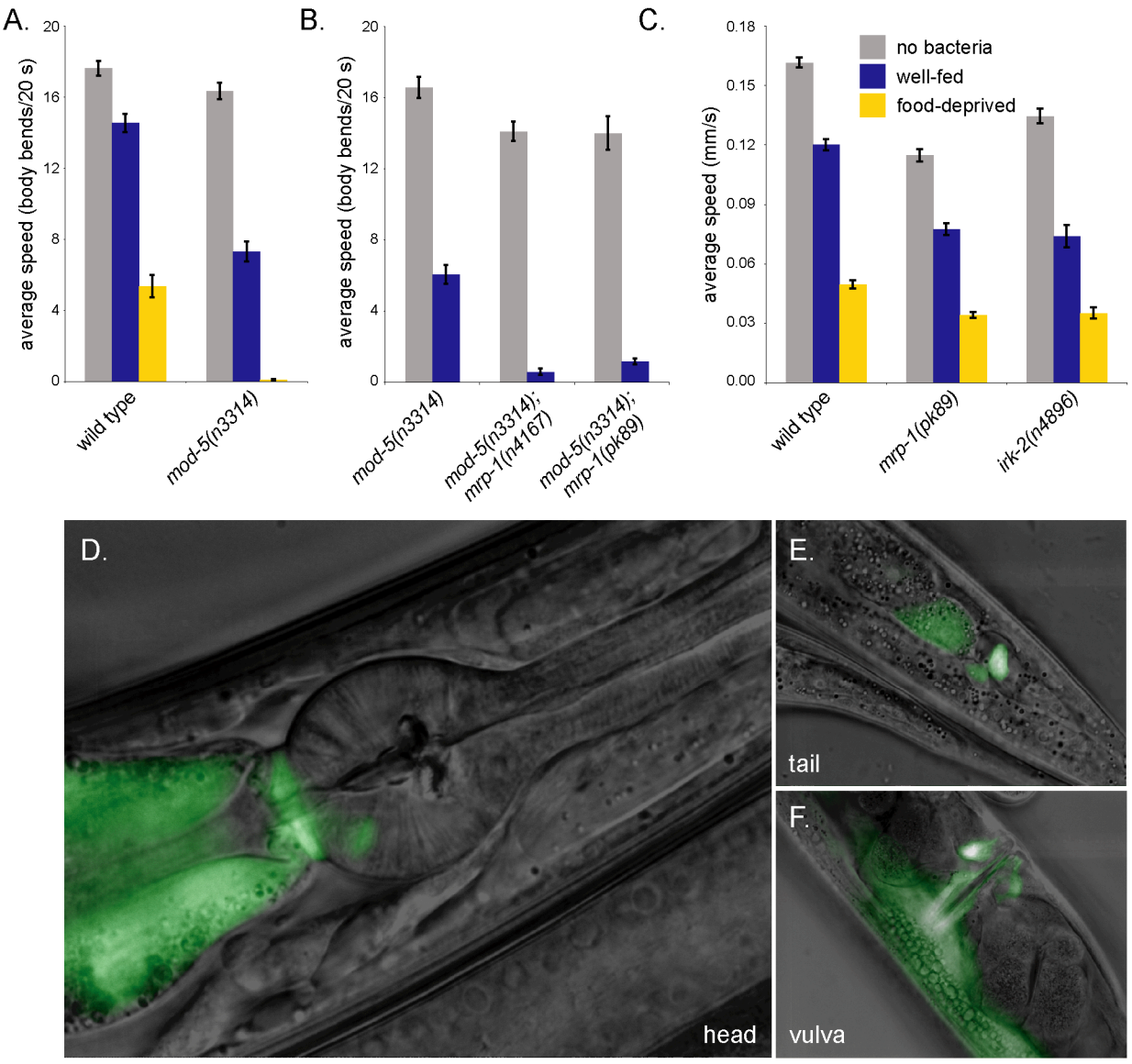

A.
B.
C.
average speed (body bends/20 s)
average speed (mm/s)
no bacteria
well-fed
food-deprived
wild type
mod-5(n3314)
mod-5(n3314); mrp-1(n4167)
mod-5(n3314); mrp-1(pk89)
mrp-1(pk89)
irk-2(n4896)
D.
E.
F.
head
tail
vulva

Figure 6.2

Mutation of the insulin receptor *daf-2* results in generally reduced locomotion and constitutive food-dependent paralysis in a *mod-5* background. Mutation of *tdc-1* also results in generally reduced locomotion. (A) Average speed of wild-type, *daf-2(e1370)*, *daf-7(e1372)*, *daf-8(e1393)*, and *daf-1(m40)* animals tested in each condition as measured by an automated locomotion tracking system. (B) Average speed of *mod-5(n3314)*, *mod-5(n3314);mrp-1(pk89)*, and *mod-5(n3314);daf-2(e1370)* animals tested in each condition as measured manually. (C) Average speed of wild-type and *tdc-1(n3420)* animals tested in each condition as measured by an automated locomotion tracking system. Error bars represent SEM. Asterisks indicate instances where the locomotion of the mutant is significantly different from wild-type locomotion in the same condition ($p <$ *.005, **.001)

Figure 6.2

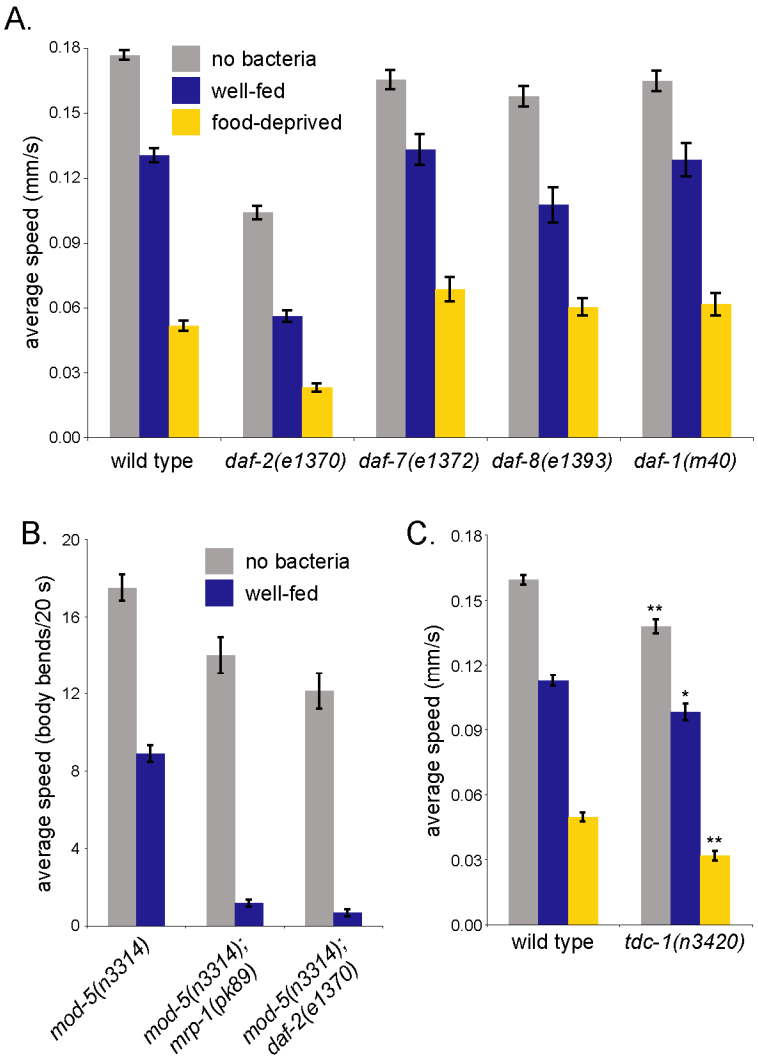

A.
no bacteria
well-fed
food-deprived
average speed (mm/s)
0.18
0.15
0.12
0.09
0.06
0.03
0.00
wild type
daf-2(e1370)
daf-7(e1372)
daf-8(e1393)
daf-1(m40)
B.
no bacteria
well-fed
average speed (body bends/20 s)
20
16
12
8
4
0
mod-5(n3314)
mod-5(n3314); mrp-1(pk89)
mod-5(n3314); daf-2(e1370)
C.
average speed (mm/s)
0.18
0.15
0.12
0.09
0.06
0.03
0.00
**
*
**
wild type
tdc-1(n3420)

Figure 6.3

The wild isolate CB4856 and *npr-1* mutants exhibit elevated locomotion on a bacterial food source. Overexpression of NPR-1 causes reduced locomotion on a bacterial food source. (A) Average speed N2 wild-type, CB4856 wild-type, *npr-1(ky13)*, and *npr-1(ok1447)* animals tested in each condition as measured by an automated locomotion tracking system. (B) Average speed N2 wild-type animals and transgenic animals carrying multiple copies of a PCR fragment containing genomic *npr-1* sequences tested in each condition as measured by an automated locomotion tracking system. Error bars represent SEM. Asterisks indicate instances where the locomotion of the NPR-1 overexpressing strain is significantly different from wild-type locomotion in the same condition ($p < *.002$)

Figure 6.3

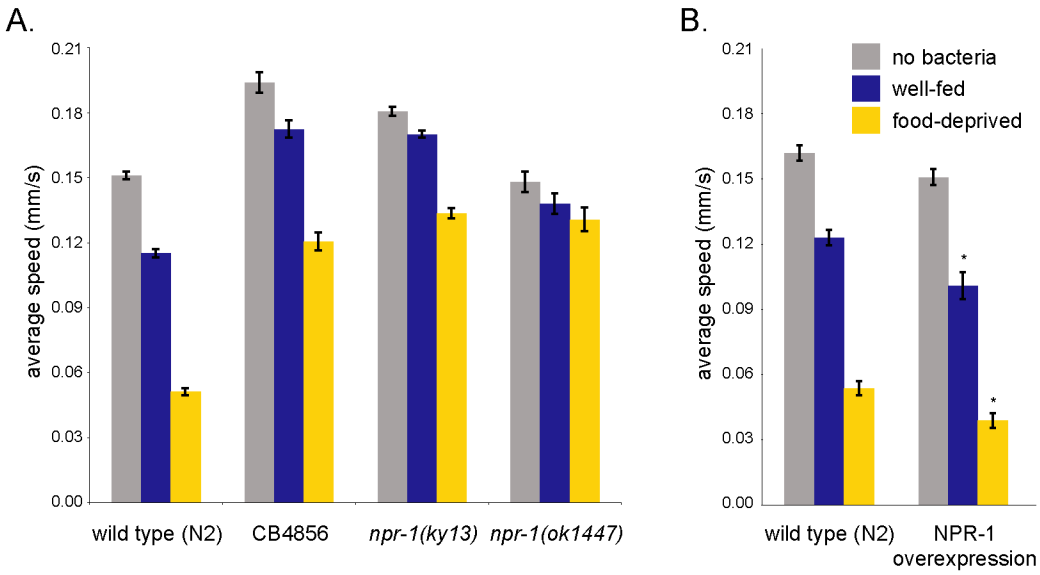

A.
B.
average speed (mm/s)
0.21
0.18
0.15
0.12
0.09
0.06
0.03
0.00
wild type (N2)
CB4856
npr-1(ky13)
npr-1(ok1447)
no bacteria
well-fed
food-deprived
*
*
wild type (N2)
NPR-1 overexpression

Figure 6.4

The elevated locomotion of *npr-1* mutants is completely epistatic the reduced locomotion of *daf-2* and *tdc-1* mutants consistent with *npr-1* acting downstream of both pathways (A) Average speed of wild-type, *daf-2(n1370)*, *npr-1(ky13)*, *daf-2(e1370);npr-1(ky13)* animals tested in each condition as measured by an automated locomotion tracking system. (B) Genetic pathway in which *npr-1* acts downstream of *daf-2* consistent with epistasis data. (C) Average speed of wild-type, *tdc-1(n3420)*, *npr-1(ky13)*, *tdc-1(n3420);npr-1(ky13)* animals tested in each condition as measured by an automated locomotion tracking system. (D) Genetic pathway in which *npr-1* acts downstream of *tdc-1* consistent with epistasis data. Error bars represent SEM.

Figure 6.4

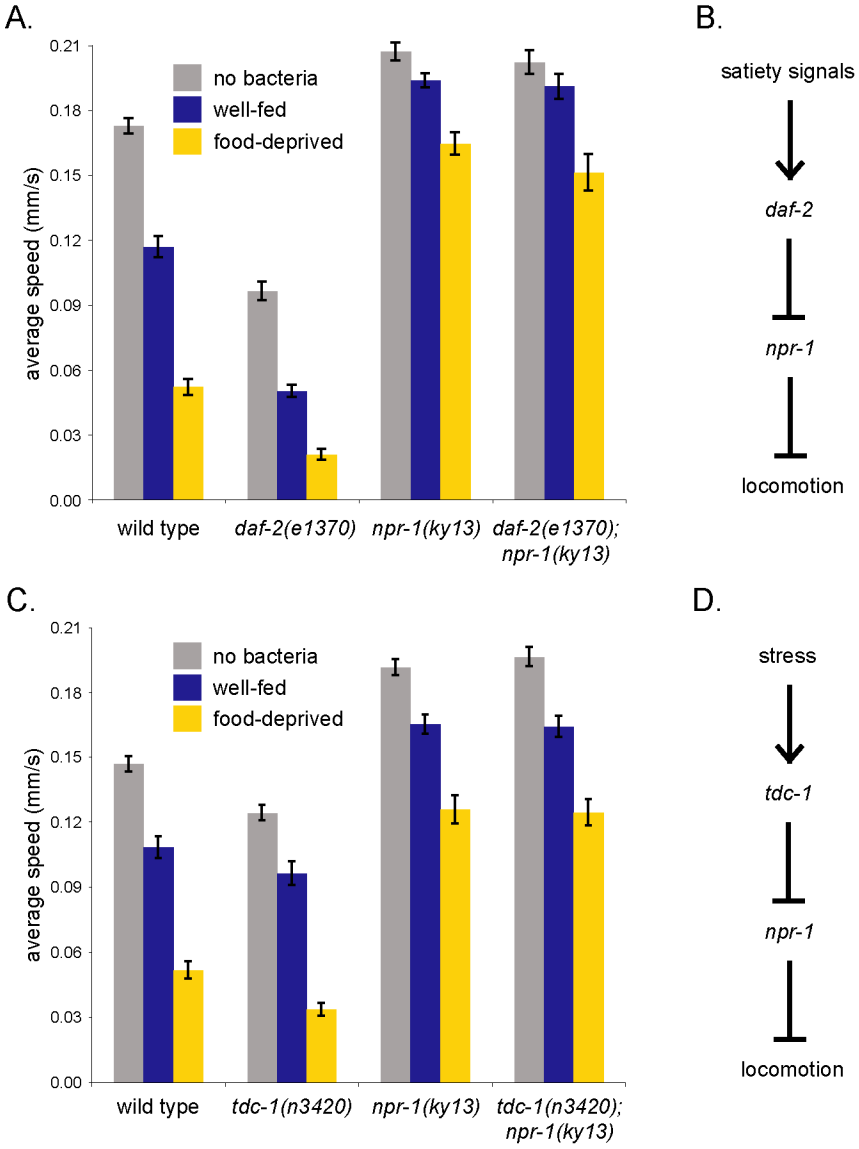

A.
average speed (mm/s)
0.21
0.18
0.15
0.12
0.09
0.06
0.03
0.00
no bacteria
well-fed
food-deprived
wild type
daf-2(e1370)
npr-1(ky13)
daf-2(e1370); npr-1(ky13)
B.
satiety signals
daf-2
npr-1
locomotion
C.
average speed (mm/s)
0.21
0.18
0.15
0.12
0.09
0.06
0.03
0.00
no bacteria
well-fed
food-deprived
wild type
tdc-1(n3420)
npr-1(ky13)
tdc-1(n3420); npr-1(ky13)
D.
stress
tdc-1
npr-1
locomotion

Figure 6.5

Mutation of the guanyl cyclase *daf-11*, cyclic nucleotide gated channels *tax-2* and *tax-4* and HSP 90 homolog *daf-21** results in reduced locomotion on a bacterial food source regardless of feeding state. Only the reduced locomotion phenotype of *daf-21** mutation is completely epistatic to the increased locomotion of *npr-1*. (A) Average speed of animals tested in each condition as measured by an automated locomotion tracking system. (B) Genetic pathway in which *daf-11*, *tax-2*, and *tax-4* act in parallel to *npr-1* consistent with epistasis data. (C) Average speed of animals tested in each condition as measured by an automated locomotion tracking system. (D) Genetic pathways in which *daf-21** acts downstream of *npr-1* consistent with epistasis data.

* Results with *daf-21* and *daf-21 npr-1* mutants were completely different upon re-thaw of *daf-21* strain and upon rebuilding and testing of the *daf-21 npr-1* double mutant. See Chapter 6 text.

Figure 6.5

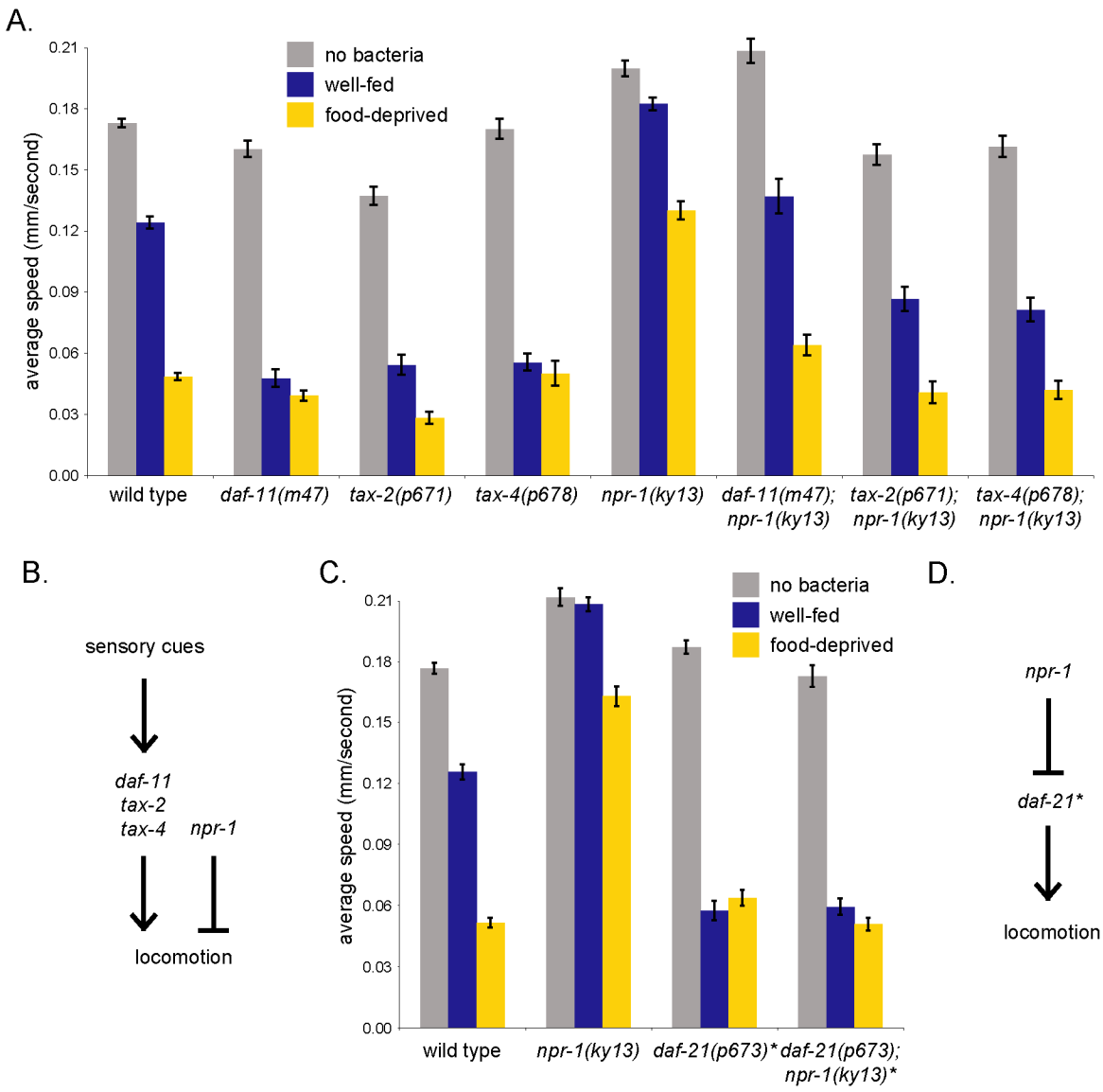
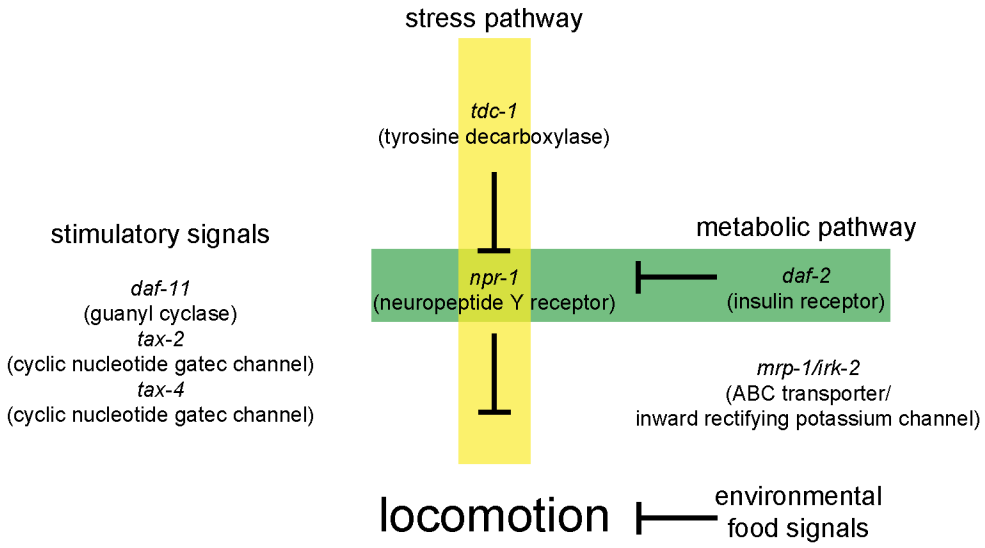

A.
no bacteria
well-fed
food-deprived
average speed (mm/second)
0.21
0.18
0.15
0.12
0.09
0.06
0.03
0.00
wild type
daf-11(m47)
tax-2(p671)
tax-4(p678)
npr-1(ky13)
daf-11(m47); npr-1(ky13)
tax-2(p671); npr-1(ky13)
tax-4(p678); npr-1(ky13)
B.
sensory cues
daf-11
tax-2
tax-4
npr-1
locomotion
C.
no bacteria
well-fed
food-deprived
average speed (mm/second)
0.21
0.18
0.15
0.12
0.09
0.06
0.03
0.00
wild type
npr-1(ky13)
daf-21(p673)*
daf-21(p673); npr-1(ky13)*
D.
npr-1
daf-21*
locomotion

Figure 6.6

Our model based on genetic epistasis experiments. We hypothesize the stress hormone octopamine and insulin-related satiety signals sensitize the animal to mechanical stimuli by suppressing *npr-1*. *npr-1* normally reduces stress responses in *C. elegans* by inhibiting an HSP90 mediated behavioral stress pathway. When *C. elegans* are well-fed, satiety signals activate *daf-2*, which partially inhibits *npr-1* partially sensitizing the animal to locomotion inducing stimuli. Octopamine/tyramine-dependent stress signals also sensitize animals to mechanical stimuli by repressing *npr-1*. When *C. elegans* are food-deprived, *npr-1* is not inhibited by *daf-2* and animals are less sensitive to mechanical stimuli. Cyclic nucleotide dependent pathways involving *daf-11*, *tax-2*, and *tax-4* might be involved in sensory processes that detect the locomotion inducing stimuli mentioned above. *mrp-1* and *irk-2* also appear to act in parallel to *npr-1*.

Figure 6.6


stress pathway
tdc-1
(tyrosine decarboxylase)
stimulatory signals
daf-11
(guanyl cyclase)
tax-2
(cyclic nucleotide gatec channel)
tax-4
(cyclic nucleotide gatec channel)
metabolic pathway
npr-1
(neuropeptide Y receptor)
daf-2
(insulin receptor)
mrp-1/irk-2
(ABC transporter/
inward rectifying potassium channel)
locomotion
environmental
food signals

APPENDIX A

An automated locomotion tracking system: Description and users guide

Our automated particle tracking system was built by Damon Clark[1] and myself and based on a system that was originally conceived of and created in the laboratory of Aravi Samuel[1]. Damon Clark[1] wrote the Matlab code for all processes and data analysis. Methods of quantifying *C. elegans* locomotion and subsequent analysis of locomotion were jointly conceived of by Damon and myself.

[1]Department of Physics and Center for Brain Science, Harvard University, Cambridge, MA 02138, USA.

Overview

To improve the way in which we study *C. elegans* locomotory behavior, we collaborated with Damon Clark in the laboratory of Aravi Samuel in the Harvard Physics Department to create an automated locomotion tracking system. Our goals were to increase the rate of data collection and remove human quantification of locomotion. We reached these goals and created a locomotion tracking system that can track the locomotion of over 100 animals simultaneously for up to10 minutes at a time at high spatial and temporal resolution.

Our system also increases the resolution at which we can study *C. elegans* locomotion and maintains the integrity of individual tracks allowing us to study the locomotion of individual animals. Although, originally designed to replace previous manual methods of scoring, this automated tracking system has also greatly extended our ability to describe *C. elegans* locomotion. We describe how this system collects, processes, and analyzes *C. elegans* locomotion data.

Physical System

Our tracking system is housed in a temperature and humidity controlled room, and is shielded from room air currents with a cloth barrier (Figure A.1). Images are acquired by a Sony XCD-710 Firewire Camera and Rainbow SGX11-II TV Zoom Lens connected to a Dell Precision 670 Workstation (Xeon 3.0GHz CPU, 3.25 GB RAM, OS: Microsoft XP Pro SP2) running Matlab version 7.0.1 with Image Acquisition and Image Processing toolboxes (Figure A.2). An array of red LEDs (Super-Red LED – RL5-R8030) was mounted on hardboard and used to illuminate our assay plates (Figure A.3). Our light array was powered by a Biopharmacia EPS60 power supply, connected with alligator clips (Figure A.2). The lids of two standard 5 cm and 10 cm *C. elegans* culture plates were spray-painted black, inverted, and used as light shields for plates in our assay. The LED light array is attached to a ring stand by a clamp that holds it 3 inches above the base of the stand. The camera is also attached to the ring-stand by an

adjustable clamp. The face of the camera lens is suspended 20 inches above the LED light array.

Data Acquisition

1. Turn on LED light array

Set the Biopharmacia power supply to a maximum voltage of 2 volts and press start.

2. Initiate the camera (only if camera is unplugged or computer is rebooted)

The camera must be initiated with third party software program named '1394CameraDemo.exe', because Matlab cannot initialize the camera after a reboot. The camera will remain initialized until it is either unplugged or the computer is turned off. To initialize camera, (a) Run '1394CameraDemo.exe', (b) check link, (c) initiate camera, (d) set frame rate to 15 fps, (e) adjust mode to 1024x768 Mono (16-bit). Steps d and e must be performed in order.

3. Run Matlab 7.0.1

4. Load *cameravid.mat* into workspace

cameravid.mat contains two objects named *sources* and *vid*. The *sources* object controls the camera settings. The following are a list of *sources* settings that were adjusted to optimize brightness and contrast for subsequent analysis. (Auto Exposure: 100, Brightness: 0, Frame Rate: 15, Gain: 390, Gain Mode: manual, Shutter: 210, Shutter Absolute: 0.009, Shutter Control: manual). The *vid* object controls video capture. The following are a list of *vid* settings that were adjusted to capture 1024x768 grayscale video at 1Hz for 120 seconds at a time. (Frame grab interval: 15, Frames per trigger: 120,

Logging mode: memory, Returned Color Space: grayscale, Time out: 350, Time Period: 1.0, Trigger Frame Delay: 0, Trigger Repeat: 0.0).

5. Set the amount of memory allocated to capturing video

>> imaqmem (400000000000);

6. Display the video

>> preview (vid);

7. Calibrate the system

Remove the lid from and place a standard (5cm) assay plate containing worms face-up in the inverted black 5cm lid on the adjustable stand below ring light. Adjust focus and zoom distance such that the horizontal visual field is 33 mm across (1024 pixels = 33 mm :: 1 pixel = approx 0.03 mm). Adjust the height of plate stand such that the surface of the agar in the assay plate is maximally illuminated when looking at the video preview. If the plate is not evenly illuminated, adjust the light array such that it is well aligned with the surface of the plate. Adjust the lens aperture such that the worms begin to display 10-50% inverted pixels (bright pixels that become black upon increasing image brightness). Recheck the adjustments.

8. Center the assay plate

Place the assay plate such that the rim of the plate is outside the camera field of view. Images are later thresholded based on maximum pixel values. If the plate is not centered, these maximally bright pixels are often at the edge of the plate rather than in worms.

9. Start recording video

>> record;

The *record* command tells the video object to start recording video for 120 seconds* capturing one frame per second. The record command immediately prompts for a user-entered filename that is used to name the video file after all images are collected. Video files contain a single object (*temp*) that contains the images collected.

Note: I have named my files with a string that contains the strain/allele names, condition (l = locomotion off bacteria, b = basal slowing condition, e = enhanced slowing condition), trial number for the day (1-4), and date. For instance, if I tested the N2 wild-type in the basal slowing condition one time on April 18, 2008, I would name the file N2b1041808. Files with the designation comp in place of the trial number and date contain pooled data from many days.

Note: When recording for the first time after starting Matlab, the counter that is displayed at the bottom left corner of the preview window often does not progress at exact one second intervals indicating that video is not being captured at exactly one second intervals. This is usually remedied by interrupting the process (*ctrl C*), stopping the video object from recording (*>> stop (vid);*), flushing data from video recording object (*>> flushdata(vid);*) and starting recording again.

*The length of recording can be adjusted by changing *vid* settings and editing *record.m* file. *vid* parameter *frames per trigger* can be changed to capture more frames. The *timeout* parameter must always be set to a value larger than *frames per trigger*. The *record.m* file must also be edited to extract more frames from the *vid* object. The line *temp = getdata (vid,120);* must be changed from *120* to the desired number of frames you have captured.

Image Processing

1. Load the video file to be processed into Matlab

2. Convert the image stack into tracks

>> [cln] = processimagessmall (ims);

processimagessmall(ims) turns a set of dark field images (*ims*) into a group of tracks (*cln*). A track is a continuous list of x,y position coordinates that represents the position of a worm on a plate. This program automates the image processing of a single file with the subprograms *getallcentroids.m*, track.*m*, *putinfields.m*, and *getcleantracks.m*. Parameters that can be adjusted in this file include *thr, mn, mx*, and *rad*, which will be explained in the description of the subprogram in which they are used below. We used the following values, for assays on 5 cm plates, which consistently gave us the longest continuous tracks: *thr = 0.09, mn = 60, mx = 140, rad = 10*. For assays on 10 cm plates, values should be scaled proportional to the camera field. *thr* values were adjusted to values between *0.06* and *0.20* for stacks of images that do not process. Generally brighter images required higher thresholds while darker images required lower thresholds.

> *getallcentroids(stk, thr, mn, mx)* takes a set of grayscale images (*stk*) and for each image subtracts away the background signal using subprogram *bgcalc.m*. It then determines centroid positions (center of mass) for each image using *getcentroids.m*. *thr, mn*, and *mx* are passed to *getcentroids.m*.
>
> > *bgcalc(stack,f)* creates a background frame that is the average of the first defined number (*f*) of frames of an image stack (*stack*). This background frame is used subsequently in *getallcentroids.m*. This background frame is subtracted from every image and leaves behind only worm signals that change from frame to frame.
> >
> > *getcentroids(im,thr,mn,mx)* takes a single grayscale image with the background already subtracted (*im*) and creates a binary image by using a

threshold value (*thr*), which is the minimum fraction of maximal pixel intensity that is considered to represent a signal. All of the continuous objects in the binary image are then dilated and eroded and all of the objects that are either smaller than a minimum (*mn*) or greater than a maximum (*mx*) number of pixels are filtered out. The centroid positions of all objects are then determined and these values are returned.

track(cents,rad) takes the centroid positions from sequential frames (*cents)* and assembles them into tracks by a method described by Crocker, et al. (1996). *track.m* uses a value (*rad)* that is the maximum distance that an object can travel between frames, to assemble tracks. *track* uses subprograms *shiftr.m*, *uberize.m*, and *unq.m*.

putinfields(in) reorganizes the output of *track.m*.

getcleantracks(in) accepts a group of tracks (*in*) and removes the tracks in which less than 10 frames are collected, splits single tracks with gaps in them of greater than 3 seconds into two tracks, removes tracks in which the total movement of the animal is less than 3 pixels, and sorts tracks by total length. The minimum number of frames collected, gap size, and minimum displacement can be adjusted internally in this file.

Automated Image Processing

1. Set the path to include directory with command files.
2. Set the current directory to the directory containing files to be processed.
3. Run *batch;*

>>*batch;* makes a list of all files in the current directory, sequentially loads each file, runs *processimagessmall.m* after loading each file, and names each object the same name as the loaded file minus the *.mat* suffix.

Analysis of Locomotion

Most of our analysis of speed contains a *frame* variable that is used to determine per/second speed measurements. To increase peak strengths in histograms, we calculated an average per/second speed by using a sliding window of a defined number of frames (*frame*). The first per/second speed measurement is calculated from the distance traveled in the first defined number (*frame*) of frames divided by the number of seconds elapsed between those frames. Our sliding windows then shifts by one second and the next calculation is made. To remove this method of speed calculation, set the window (*frame*) to 1. We considered using both point-to-point and the path traveled by the animal to measure distance in our calculation of average speed. Point-to-point determination of distance is the vector distance between the start and end point divided by the time it took to travel that distance. While this appeared to be the best way to eliminate the noise of centroid displacement, it also eliminated much of the signal, since animals do not always move in a single direction over a defined period of time. We therefore opted to use the path that the animal travels divided by the time it took to travel that distance for determination of per/second speeds. Though a bit of a misnomer, we often refer to per/second speed rates calculated in this fashion as the instantaneous speeds of animals.

Calculating and extracting speed from tracks

>> *makevellisttrack(in,fr)* accepts a group of tracks (*in*) and returns a list of independent speeds recorded for each track using a sliding window of defined size (*fr*).

>> *ave_list(din,FR,MINF)* accepts a group of tracks (*din*), calculates speeds using a sliding window of defined size (*FR*) and the path method of calculating distance, filters out tracks of length less than a defined (*MINF*) number of frames, and returns a list of average track speeds.

>> *out=computespeed(in,window);* accepts a group of tracks and adds speed calculations to each track using a sliding window of defined size (*window*), and then returns this modified set of tracks (*out*).

Determining average speeds of tracks

>> *getmean(ims,fra)* accepts a group of tracks (*ims*), calculates the instantaneous speed measurements of all animals using a sliding window of defined size (*fra*), and then returns the average, standard deviation, and standard error of the mean of all instantaneous speed measurements in the group. *fra* is included because the size of the sliding window can affect what data is included in this calculation.

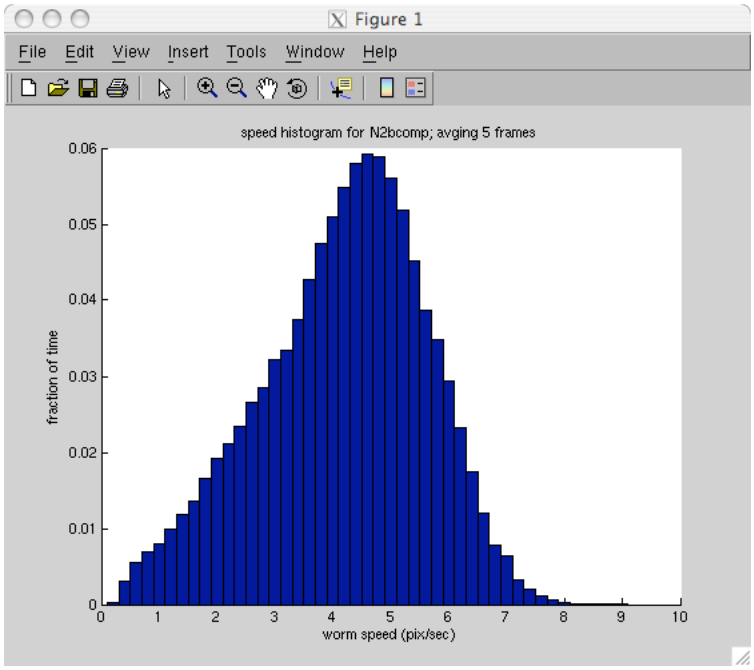
>> *getmean_ave(ims,fra)* accepts a group of tracks (*ims*), calculates the instantaneous speed measurements of all animals using a sliding window of defined size (*fra*), filters out tracks shorter than 10 frames, calculates the average speed of every track, and returns the average of average speeds of all tracks, the standard deviation, and the standard error of the mean of the average of average track speeds. *fra* is included because the size of the sliding window can affect what data is included in this calculation.

Visualizing speed of groups of animals

>> *plotvelhistsm(ims,frm)* accepts a group of tracks (*ims)*, calculates the instantaneous speed measurements of all tracks using a sliding window of defined size (*frm*), and plots an histogram of instantaneous speed recordings.

>> *plotvelhistsm_frac(ims,frm)* is identical to *plotvelhistsm(ims,frm)* except data is expressed as a fraction of total speed measurements.

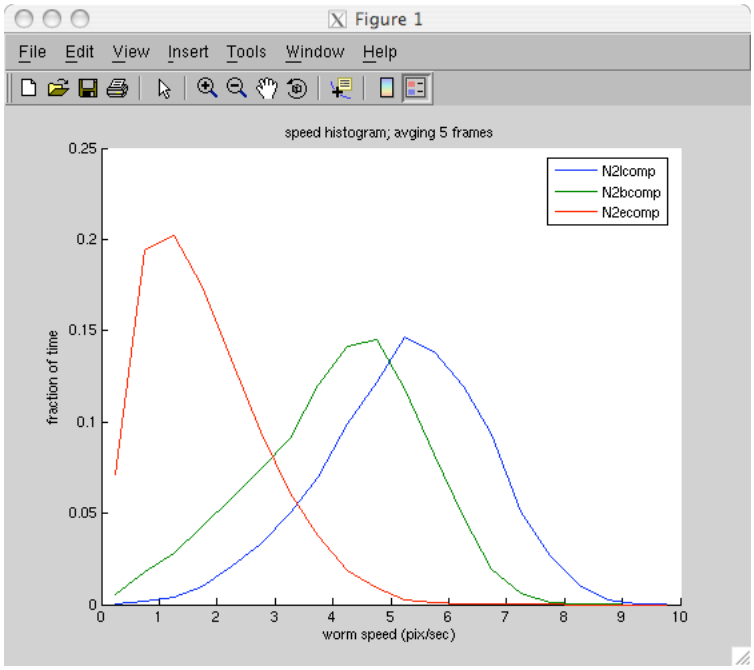
Example:
>> *plotvelhistsm (N2bcomp,5);*



>> *plotmvelhists(frm,binning,varargin)* accepts multiple groups of tracks (*varargin*), *varargin* is structure (a,b,c,…), calculates the instantaneous speed measurements of all animals using a sliding window of defined size (*frm*), and creates outlines of histograms of instantaneous speed recordings for each group of tracks. Data is grouped and plotted at defined (*binning*) intervals.

Example:

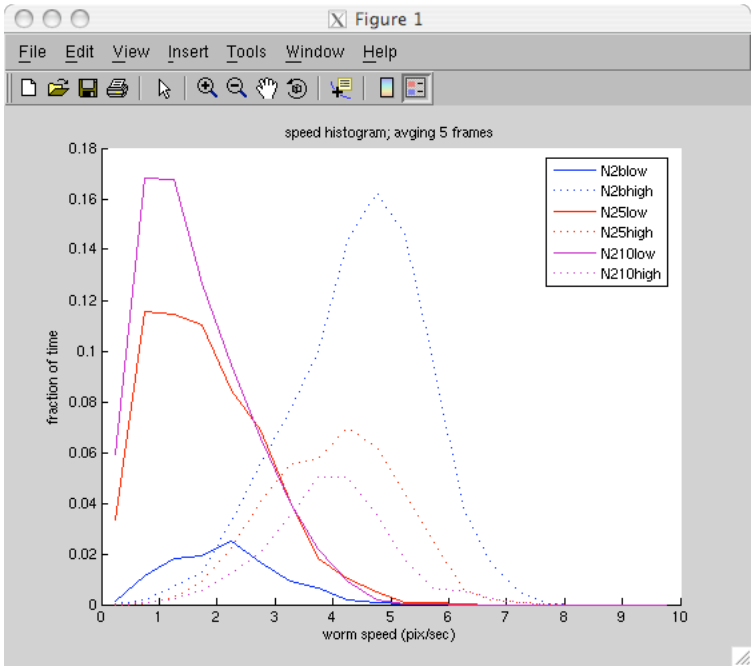
>> *plotmvelhists (5,5,N2lcomp,N2bcomp,N2ecomp);*



>> *plotmvelhists_paired(frm,binning,varargin)* accepts multiple pairs of tracks (*varargin*), *varargin* structure is (a1, a2, b1, b2…), calculates the instantaneous speed measurements of all tracks using a sliding window of defined size (*frm*), and creates outlines of paired histograms of instantaneous speed recordings from each pair of tracks (a1, a2) such that the fraction of speed recordings in each member of the pair are plotted with respect to the total number of speed recordings in the pair. Pairs are color-coded. The first in the pair is represented by a solid line and the second is represented by a dotted line. Data is grouped and plotted at defined (*binning*) intervals.

Example:

>> *plotmvelhists_paired(5,5,N2blow,N2bhigh,N25low,N25high,N210low,N210high)*

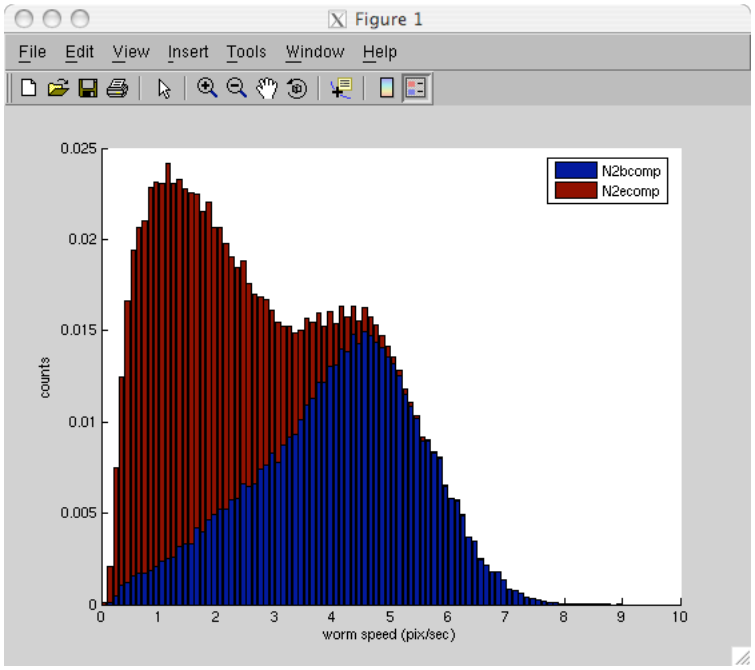


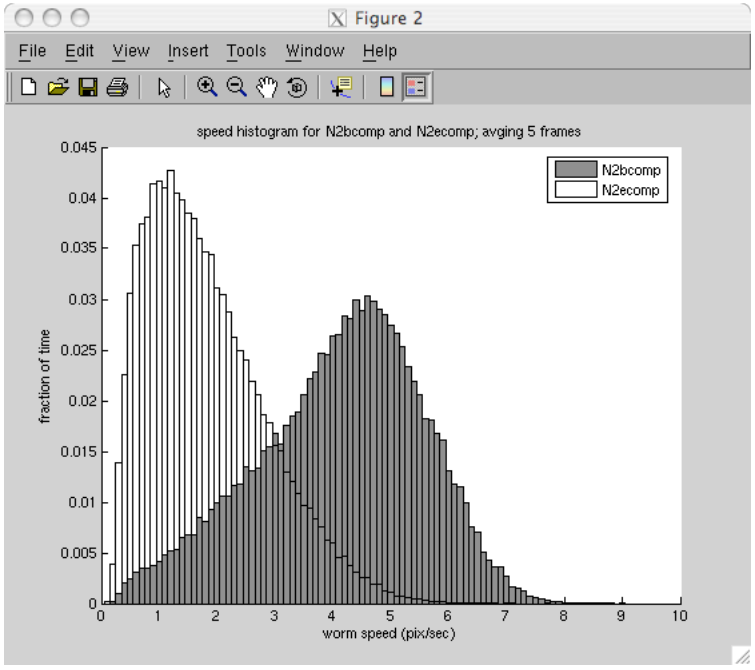
>> *plotavehistsm_frac(ims,frm)* accepts a group of tracks (*ims*), calculates the instantaneous speed measurements of all tracks using sliding window of defined size (*frm*), determines the average speed of each track, filters out tracks of less than 10 frames and plots a histogram of all average track speed measurements as a fraction of total tracks.

>> *compare_distributions(f1,f2,frm)* accepts two groups of tracks (*f1* and *f2*) calculates the instantaneous speed measurements of all tracks using a sliding window of defined size (*frm*), and creates two separate histograms of instantaneous speed measurements of each of the two groups. The first with color-coded data added together in each bin and the second with data from both overlaid.

Example:

>> *compare_distributions(N2bcomp,N2ecomp,5)*

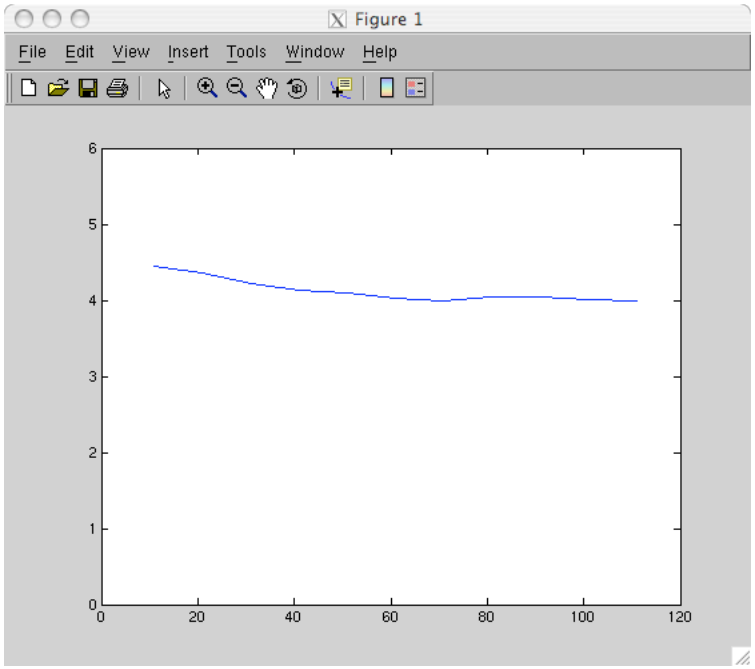




>> *plotveltime(in,fr,window)* accepts a group of tracks (*in)*, calculates the instantaneous speed measurements of all animals using a sliding window of defined size (*window*), and plots the average speed of animals in the group at intervals as the assay progressed over a specified assay period (*fr*). *fr* input is structured (beginning time : interval size : end time).

Example:

>> plotveltime (N2bcomp,1:10:120,5)

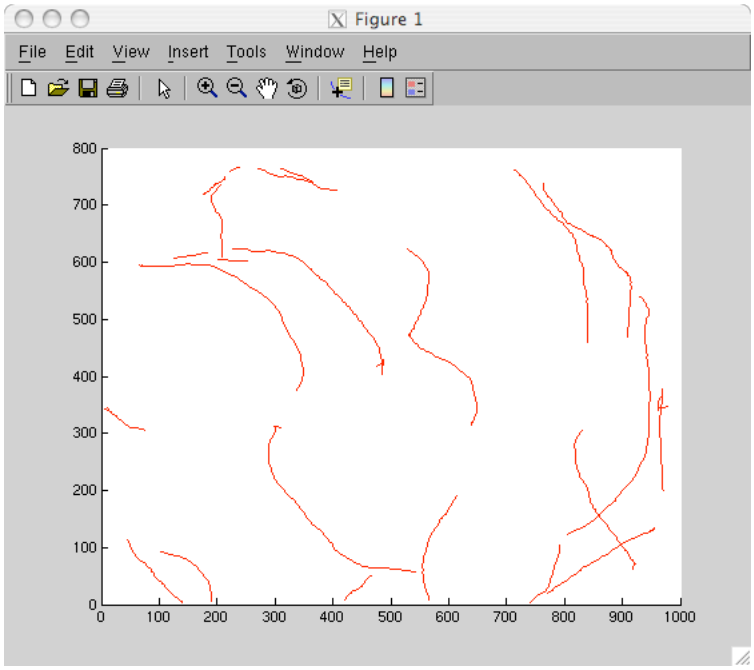


Visualizing properties of individual tracks

>> plotcentroids(trf) accepts a group of tracks (*trf*) and plots all of the tracks in x,y coordinate space.

Example:

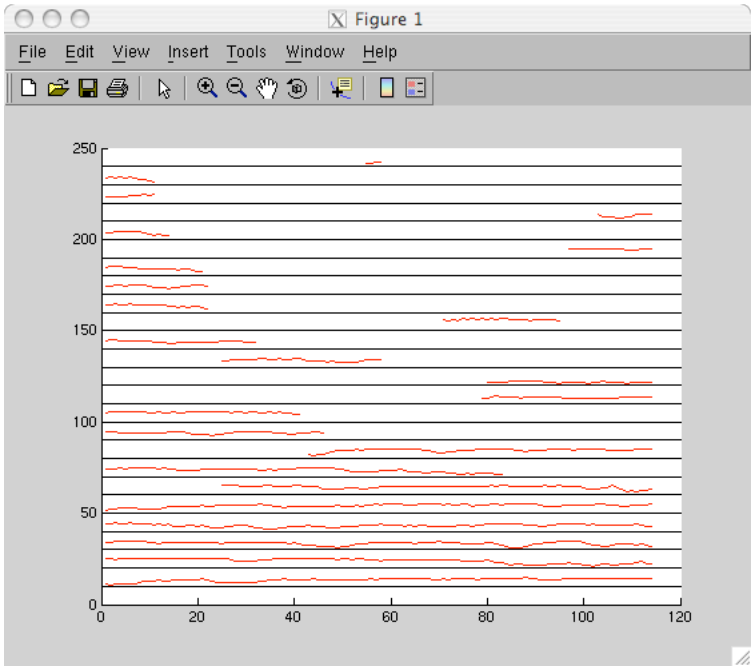
>> plotcentroids (N2b1102807)



>> *trackstacksm(tr,fi)* accepts a group of tracks (*tr*), adds instantaneous speed measurements to all tracks using a 5 second sliding window, filters out the tracks that are shorter than a defined number of (*fi*) frames and separately plots the speed recordings of all individual animals as a function of time in different rows of same figure. Each row is 10 pixels/second in height.

Example:

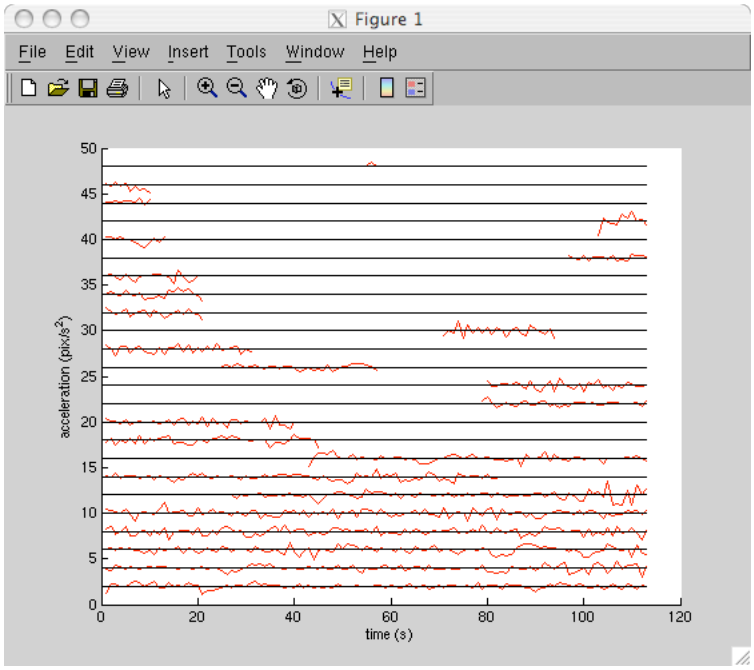
>> *trackstacksm (N2b1102807,5)*



>> *trackstacksm_acceleration(tr,fi)* accepts a group of tracks (*tr*), adds instantaneous speed measurements to all of the tracks using a 5 second sliding window, filters out the tracks that are shorter than a defined (*fi*) number of seconds, and separately plots the acceleration of all of the individual animals as a function of time in different rows of the same figure. Each row is 2 pixels/second/second in height.

Example:

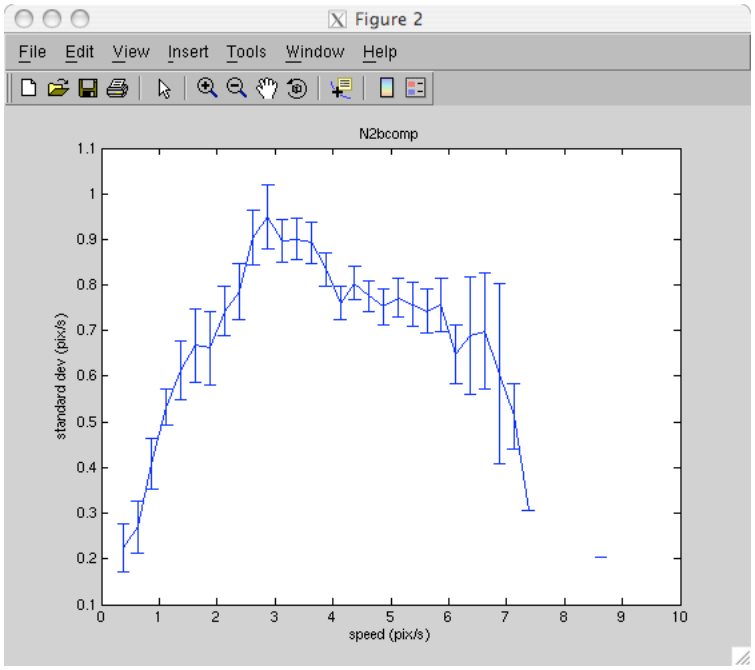
>> *trackstacksm_acceleration(N2b1102807,5)*

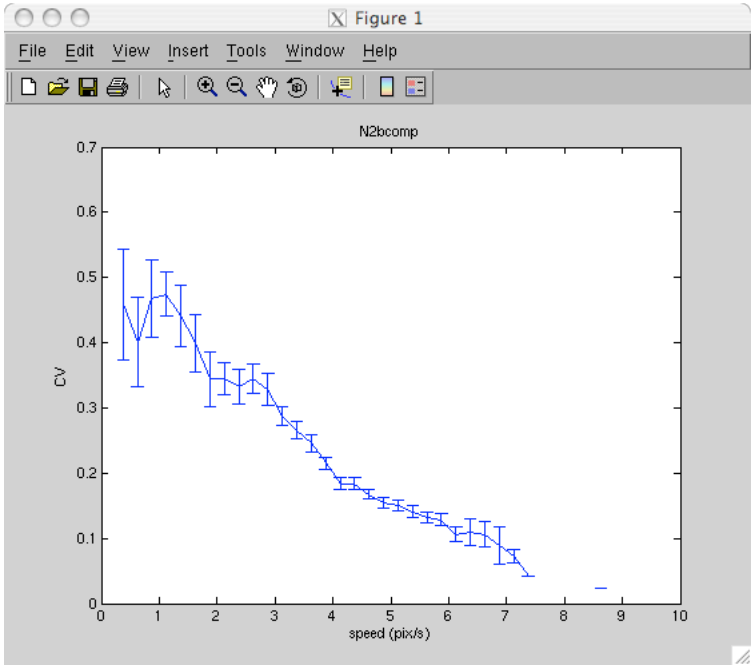


>> *cv_dist(tr,fi)* accepts a group of tracks (*tr*), calculates the instantaneous speed measurements of all of the animals using a 5 second sliding window, filters out the tracks that are less than a defined (*fi*) number of frames, splits the tracks into bins by average track speed, calculates the standard deviation and coefficient of variation (standard deviation/average speed) of speed measurements within each track, and plots the average standard deviation and the coefficient of variation of intra-track speed measurements, that are first weighted by track length, for each bin vs. average speed of tracks.

Example:

>> *cv_dist(N2bcomp,5)*





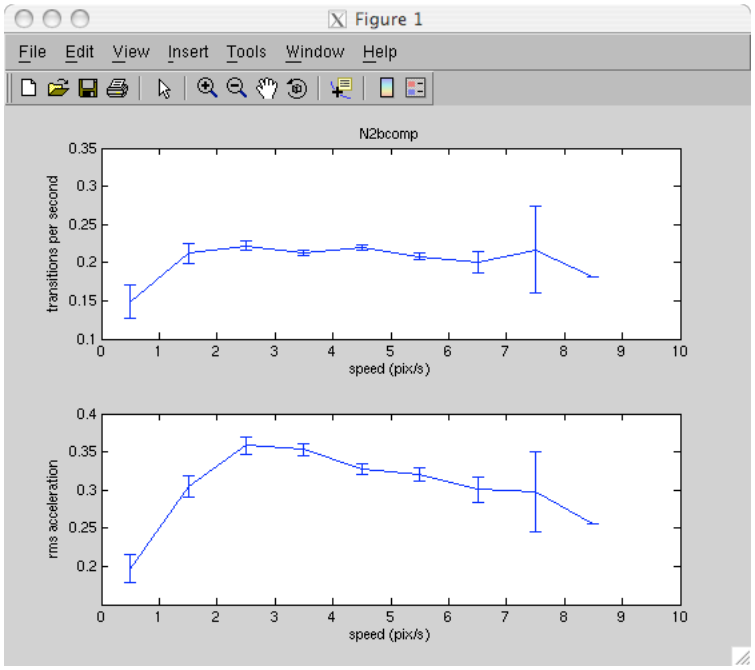
>> *cv_distfil(tr,fi)* is same as *cv_dist(tr,fi)*, with the exception that only the bins with more than one track in them are included in plot.

>> *speedshiftevents(tr,fi)* accepts a group of tracks (*tr*), calculates the instantaneous speed measurements of all animals using a 5 second sliding window, calculates the instantaneous acceleration measurements of all animals, filters out the tracks that are

less than a defined (*fi*) number of frames, splits the tracks into bins by average track speed, calculates the number of acceleration events that exceed a certain threshold (*ATHRESH*), calculates the root mean square of acceleration of each track, and then creates two plots. The first plot is the average number of acceleration events per second that exceed the threshold, weighted by track length vs. the average speed of tracks in each group. The second plot is the average root mean square of acceleration per second for each bin vs. the average speed of tracks in each group.

Example:

>> *cv_dist(N2bcomp,5)*



>> *speedshifteventsfil(tr,fi)* is same as *speedshiftevents.m*, with the exception that only bins with more than one track in them are included in plot.

Sorting and filtering groups of tracks

>> *[trout]=track_sorter(tr,method)* accepts a group of tracks (*tr*), determines the speed of tracks, sorts them according to the sort parameter (*method*), and returns the sorted

tracks (*trout*). *method* can be the length of the track ('length'), mean speed of the track ('mean'), or standard deviation of speeds for the track ('std').

>> *[filtered]=filter_size(din,tracklength)* accepts a group of tracks (*din*) and returns the tracks (*filtered*) that are longer than a defined number of frames (*tracklength*).

>> *[d1,d2]=split_data(din,thresh,opt)* creates two groups (*d1* and *d2*) that contain the tracks (*din*) that have been sorted by a parameter of track speed (*opt*) using a threshold value (*thresh*) . *opt* can be *min*, *max*, *mean*, or *med*.

Acknowledgments

I would like to thank Damon Clark for his technical wizardry, stimulating conversations, and patience in explaining to me the complicated statistical analysis he proposed to study *C. elegans* locomotion. I also thank Aravi Samuel for inspiring the vision and desire to pursue a technology that completely changed the way we think about and study the modulation of *C. elegans* locomotion.

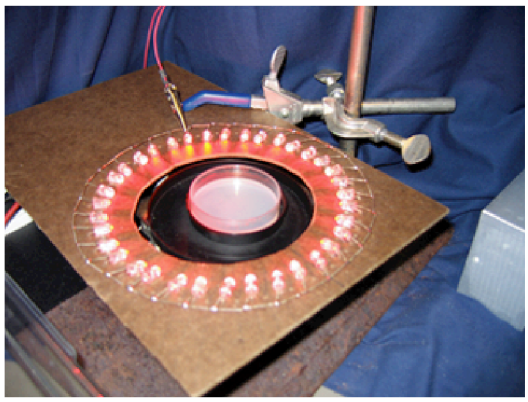
Reference

CROCKER, J. C., GRIER, D. G., 1996 Methods of Digital Video Microscopy for Colloidal Studies. Journal of Colloid and Interface Science **179:** 13.

Figure A.1

The Wormtracker

Mounted Camera and Light Array



LED Light Array

Figure A.2

Diagram: Wormtracker

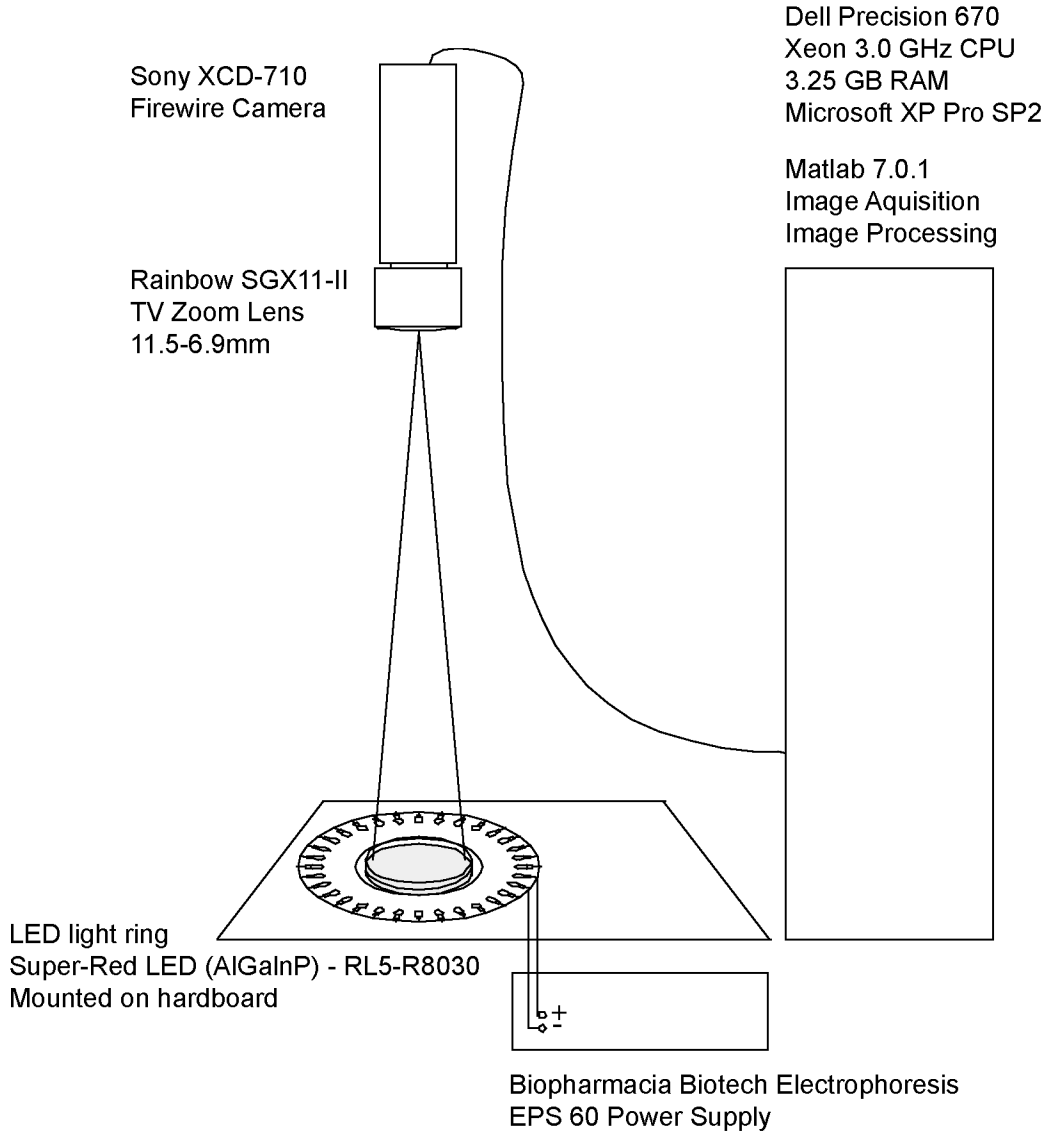
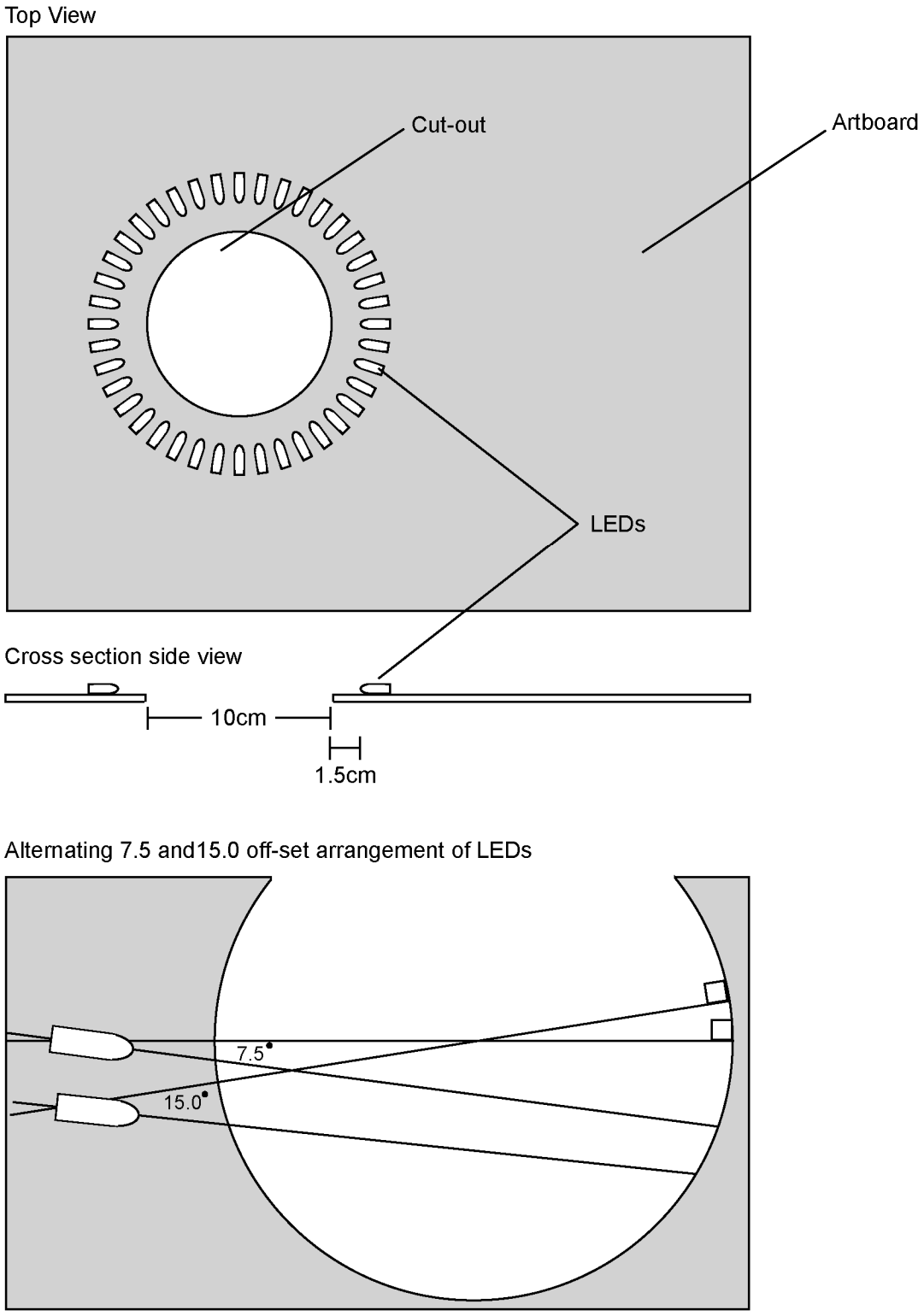


Figure A.3

Diagram: Ring Light



APPENDIX B

Genetic screens for *mod-5* suppressors and enhancers

Introduction

Beth Sawin, a previous Horvitz laboratory graduate student, described an assay in which the locomotion rates of well-fed and food-deprived *C. elegans* hermaphrodites on a bacterial lawn are very different depending on whether or not they have been previously food deprived (SAWIN *et al.* 2000). A bacterial food source generally suppresses *C. elegans* locomotion. In the Mod Assay, Beth found that upon contact with a bacterial food source, well-fed animals slow a little while food-deprived animals slow much more (SAWIN *et al.* 2000) (Figure B.1). These two different responses to a bacterial food source were named the basal and enhanced slowing responses, respectively (SAWIN *et al.* 2000).

To identify genes involved in further reducing the locomotion of animals on bacteria after food deprivation, Beth Sawin performed a genetic screen for mutants that exhibit reduced enhanced slowing (SAWIN *et al.* 2000). Mod mutants isolated in Beth Sawin's original screen proved difficult to clone. We hoped to simplify the cloning process by identifying additional mutants in a conceptually identical screen performed in a sensitized genetic background. Mutations in the *C. elegans* serotonin reuptake transporter *mod-5* cause animals to exhibit a serotonin-dependent paralysis upon entering a bacterial lawn only in the food-deprived state (SAWIN *et al.* 2000; RANGANATHAN *et al.* 2001). In this background we performed a *mod-5* suppressor screen for mutants that failed to become paralyzed upon entering a bacterial lawn in the food-deprived state. Generally we hoped to identify mutants that behave as if they are well-fed even when they are food-deprived.

My initial interest in studying this modulated behavior in *C. elegans* was to explore the molecular and cellular mechanism by which *C. elegans* generates and stores information about its food-deprived state and uses this information to modulate its behavior. Inspired by human satiety signaling we proposed that animals with defects in satiety signaling might exhibit greater slowing not only after food-deprivation but also in the well-fed state. We refer to this screen as a *mod-5* enhancer screen. In a complimentary screen, we looked for mutants that exhibited food-dependent paralysis, typical of food-deprived *mod-5* mutants, but in the well-fed state. Generally we hoped to

identify mutants that behave as if they are food-deprived even when they are well-fed. In this chapter we report the identification and preliminary characterization of mutants isolated in both of these screens.

A screen for suppressors of *mod-5(n3314)*

To look for mutants that exhibit well-fed behavior even when food-deprived, we performed a *mod-5(n3314)* suppressor screen for mutants that no longer become paralyzed upon entering a bacterial lawn in the food-deprived state. *mod-5(n3314)* mutants are also hypersensitive to paralysis by exogenous serotonin and concurrently, Megan Gustafson, another Horvitz laboratory graduate student, performed screens to identify suppressors of *mod-5(n3314)* serotonin hypersensitivity (GUSTAFSON 2007). We therefore also tested our mutants for suppression of serotonin hypersensitivity.

In this screen we used EMS to mutagenize *mod-5(n3314)* adult hermaphrodites. Three mutagenized parental (P_0) adults were transferred to each plate and grown for four days at 20°C until the plate became full of adult animals. Plates were combined in a pair-wise fashion such that 44 plates of approximately 300 adult progeny (F_1) were consolidated into 22 groups of 600 adult progeny. These 22 groups were then bleached separately and developmentally synchronized in liquid S-medium. Approximately 300 synchronized progeny (F2) from each group were grown up on plates to adulthood and approximately 150 animals per plate were screened in our assay. In the screen for *mod-5(n3314)* suppression, mutagenized animals were food deprived in liquid for 30 minutes and transferred to large plates containing a ring shaped bacterial lawn. Animals were allowed migrate into the bacterial lawn for 10 minutes and animals that were not paralyzed at the inner edge of the bacterial lawn were picked as potential *mod-5* suppressor mutants (Figure B.2).

After screening through approximately 1650 mutagenized haploid genomes, 45 candidate suppressors were isolated. Four were sterile and 18 retested and were characterized. These isolates exhibited various levels of suppression with respect to modulation of locomotion and serotonin sensitivity defects (Figure B.3A). Based on

phenotype and the groups from which they were isolated, at least 12 independent mutations were isolated that suppress the *mod-5(n3314)* modulation of locomotion defect (Figure B.3B). We hypothesize that the *mod-5(n3314)* suppressor mutations might lie in genes that are involved in hunger signaling, serotonin signaling, the detection or transduction of bacterial cues that suppress the locomotion of *C. elegans*, or suppression of *C. elegans* locomotion generally.

A screen for enhancers of *mod-5(n3314)*

To look for mutants that exhibit constitutive food-deprived behavior we performed a *mod-5(n3314)* enhancer screen for mutants that become paralyzed upon entering a bacterial lawn in the well-fed state as well as in the food-deprived state. In this fashion we hoped to identify genes that are involved in signaling the well-fed state in *C. elegans*. Since reduced locomotion on a bacterial food source might be caused mutation of any number of genes involved in coordinating *C. elegans* locomotion we required that our isolates move well in the absence of a bacterial lawn. Two different mutagens were used in two identical screens.

In our first screen *mod-5(n3314)* animals were mutagenized with EMS and treated as described in our *mod-5* suppressor screen. Mutagenized animals were washed free of bacteria and immediately transferred to large plates containing a ring-shaped bacterial lawn. Animals were allowed migrate into the bacterial lawn for 30 minutes and animals that remained paralyzed at the inner edge of the bacterial lawn were picked as potential *mod-5* enhancer mutants (Figure B.4). In an EMS mutagenesis screen of 5250 mutagenized genomes 278 candidates were picked and 33 retested and were stored as frozen strains.

To ensure that suppressors were not generally defective in the ability to move, we required that mutants not appear uncoordinated the absence of a bacterial lawn and that the food-dependent paralysis could be antagonized by the drug methiothepin (Figure 2.5A). Methiothepin is a serotonin and dopamine receptor antagonist (MONACHON *et al.* 1972; DALL'OLIO *et al.* 1985) that was shown to increase the locomotion of animals on a

bacterial lawn after food-deprivation (SAWIN *et al.* 2000). Only mutants that moved well in the absence of bacteria and whose food-dependent paralysis was suppressed by methiothepin were further characterized.

To ensure we were not isolating animals that were actually food-deprived, we required that our candidates grow at a normal rate, which likely indicates the normal acquisition and absorption of nutrients. To determine growth rate, animals were picked at the L4 larval stage and the time between when they were picked and the time at which they became gravid (full of eggs) adults was reported. Since *C. elegans* only lays eggs on a bacterial food source (HORVITZ *et al.* 1982), we examined the egg-laying behavior of isolates. Our thought was that the detection of ingested bacteria might stimulate egg-laying and mutants defective in this process might retain eggs. To determine egg-laying behavior, we bleached multiple animals, a process that releases eggs from the animal, and reported the average number of eggs retained per animal for each strain. Finally it was noted that mechanosensory-defective mutants (CHALFIE *et al.* 1985) exhibit sluggish locomotion on a bacterial food source, so we tested our isolates for mechanosensory defects as well. To test for sensitivity to mechanical touch we gently touched an eyelash across the body of the animal immediately behind the posterior bulb of the animal's pharynx. This treatment causes forward moving animals to reverse their locomotion. We reported the frequency with which each strain reverses in response to this mechanical stimulus. These results are summarized in Figure B.5.

Six mutants had a strong constitutive food-dependent paralysis phenotype that was antagonized by methiothepin and were not mechanosensory defective. Genetic complementation tests were performed with these strains that contained *n3924*, *n3925*, *n3948*, *n4018*, *n4021*, and *n4022*. *n4017* and *n4019* were not included in complementation tests because *n4017* was mechanosensory defective and *n4019* laid eggs constitutively (TRENT *et al.* 1983). Of these alleles tested, only *n3925* and *n4022* failed to complement for the constitutive food-dependent paralysis phenotype and likely contain mutations in the same gene. The constitutive food-dependent paralysis phenotype of *mod-5(n3314); n3924* mutants showed genetic linkage to *bli-6* on chromosome IV and *mod-5(n3314); n4019* showed genetic linkage to *dpy-5* on chromosome I. Subsequent work by a Horvitz laboratory post-doctoral researcher Allan

Froelich determined that *n3925* and *n4022* were alleles of C44B9.1, which encodes a novel coiled coil-containing protein.

To facilitate the cloning of mutants that exhibit constitutive food-dependent paralysis in a *mod-5* background, we continued screening using a Mos1 transposon mutagenesis protocol as described by Boulin et al (2007). Mos1 is a *Drosophila* transposable element (JACOBSON *et al.* 1986). Once a gene is mutagenized using this method, the transposon tagged gene can be identified by isolating genomic DNA, digesting the genome, circularizing fragments with a ligation reaction, and amplifying the sequences external to the transposon by PCR. In a screen of 20,375 transposon-mutagenized genomes, 1,114 candidates were picked and 10 retested and were stored as frozen strains. Of the original ten isolates, we were able to backcross only five: *n4158, n4159*, *n4161*, *n4164*, and *n4167*. Of these mutants, the paralysis of *n4159*, *n4161*, *n4164*, and *n4167* containing strains were suppressed by methiothepin. We attempted to identify the genes that had been mutagenized in the five backcrossed strains using PCR and found that the genome of only one of the five strains contained a Mos1 transposon. The reason we did not identify transposon sequences in these Mos1 mutagenized animals is likely due to re-excision of the Mos1 transposons.

A single Mos1 transposon insertion defined the *n4167* mutation and lies in the gene *mrp-1*, which encodes an ABC transporter. Further characterization of this mutant is detailed in Chapter 6.

Acknowledgments

I would like to thank Megan Gustafson. Together we generated *mod-5* Mos1 transposon mutagenized animals for our respective genetic screens. I thank Allan Froelich for sharing his unpublished results.

References

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Figure B.1

Well-fed and food-deprived animals exhibit different rates of locomotion on a bacterial food source. Average speeds of animals in the absence of bacteria, well-fed in the presence of bacteria, and food-deprived in the presence of bacteria are shown. Error bars represent SEM.

Figure B.1

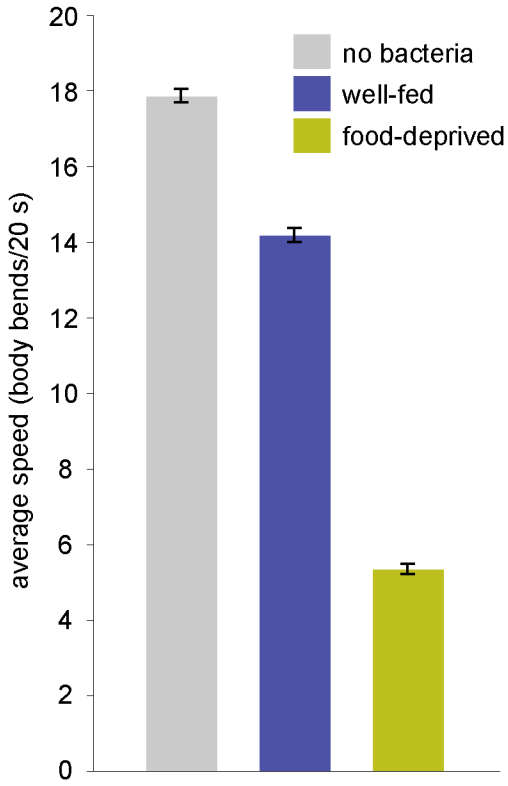

20
18
16
14
12
10
8
6
4
2
0
average speed (body bends/20 s)
no bacteria
well-fed
food-deprived

Figure B.2

mod-5 suppressor screen outline. *mod-5* mutant animals are mutagenized with EMS and grown for two generations. The F2 generation is food-deprived for 30 minutes and transferred to the middle of a ring-shaped bacterial lawn. Animals are allowed to migrate for 10 minutes and animals that are not immobilized at the inner ring of the bacterial lawn are picked as candidate suppressors.

Figure B.2

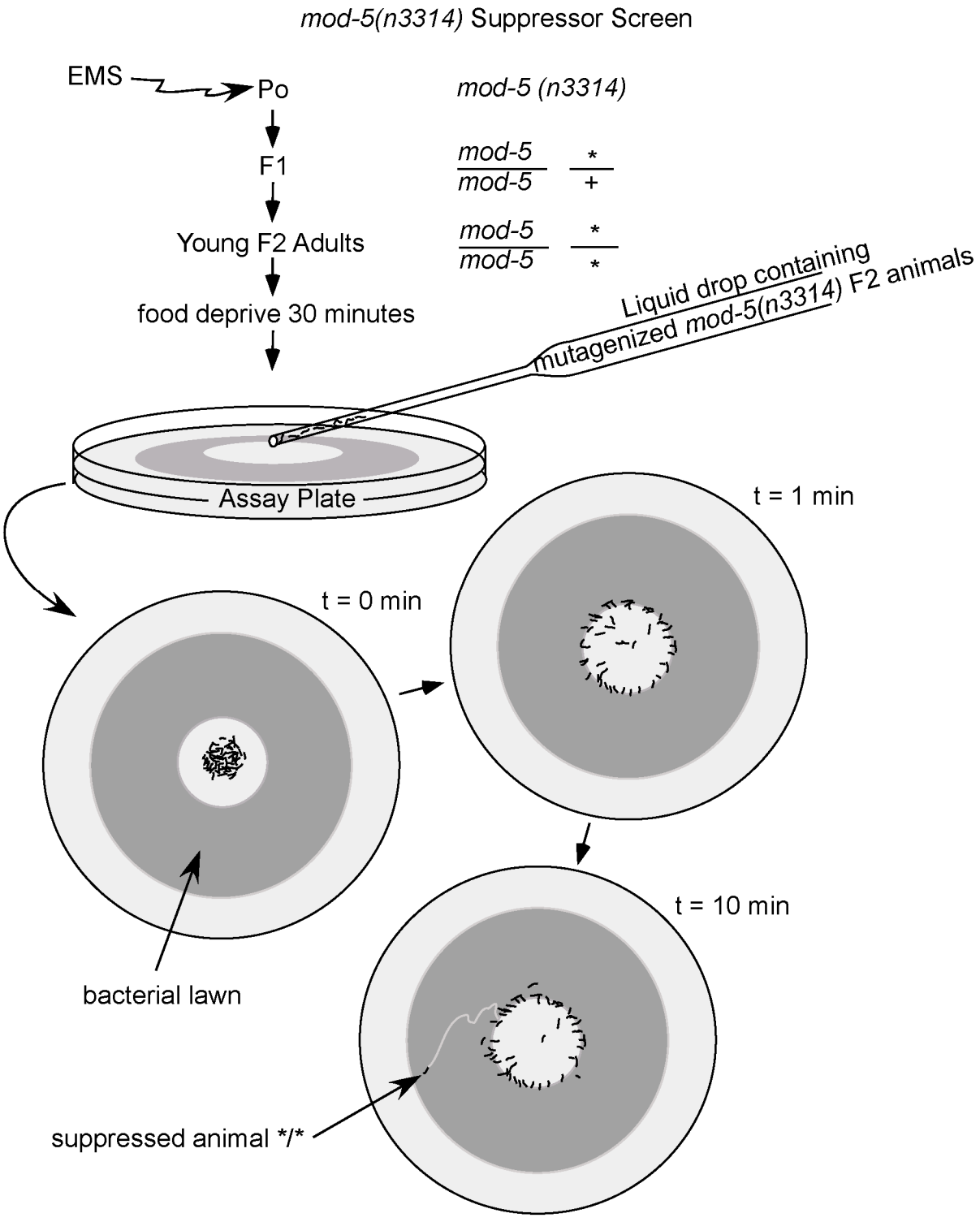
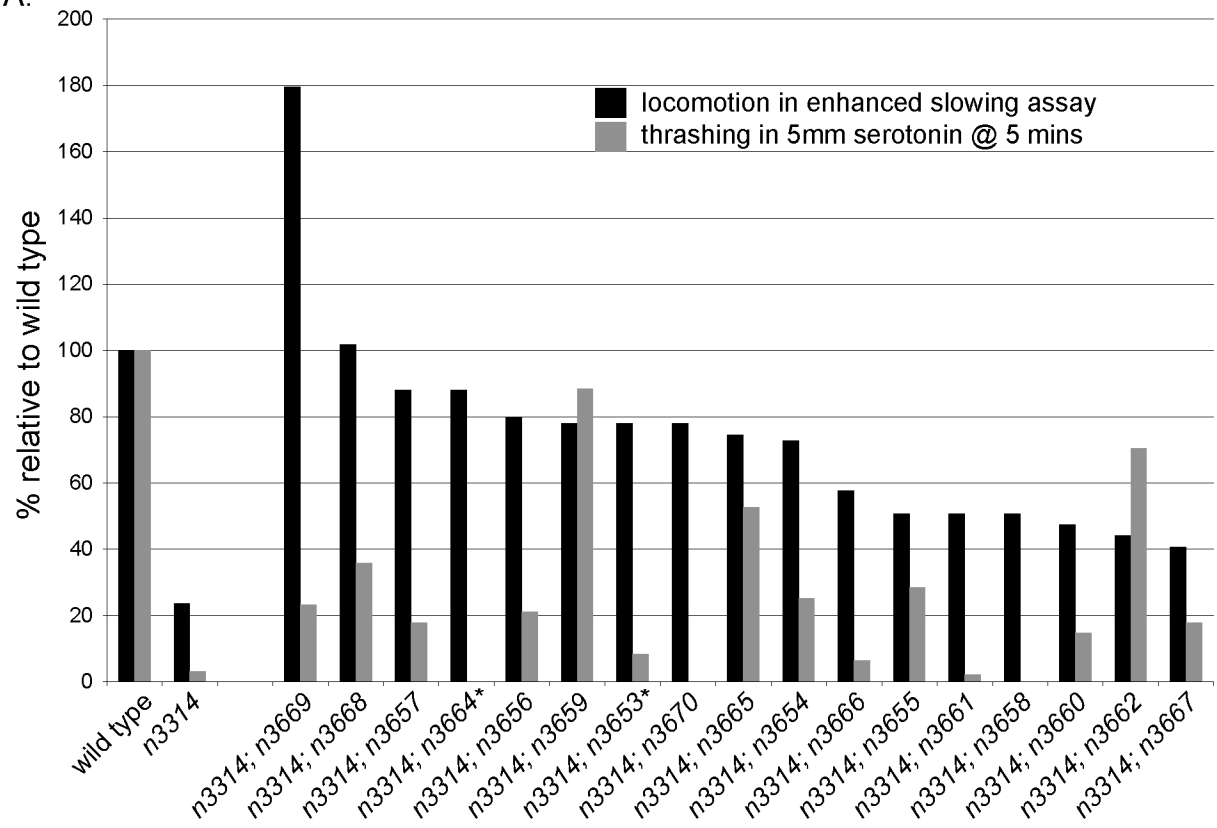

mod-5(n3314) Suppressor Screen
EMS
Po
F1
Young F2 Adults
food deprive 30 minutes
mod-5 (n3314)
mod-5 / mod-5 * / +
mod-5 / mod-5 * / *
Liquid drop containing mutagenized mod-5(n3314) F2 animals
Assay Plate
t = 0 min
t = 1 min
t = 10 min
bacterial lawn
suppressed animal */*

Figure B.3

Summary of *mod-5* suppressor screen isolate phenotypes. (A) Enhanced slowing locomotion and serotonin sensitivity phenotypes relative to wild type. Serotonin sensitivity data reflects the fraction of animals swimming in 5mm serotonin after 5 minutes of exposure. (B) Isolates obtained from the same isolation plate and that exhibit a similar phenotype might have come from the same parental clone and likely contain identical mutations.

Figure B.3

A.



*n3314; n3663** data not recorded
* asterisked mutants do not swim well in the absense of serotonin

B.

Isolates from the same plate and similar phenotypes

Isolation Plate	Alleles
1	*n3653, n3654*
3	*n3655, n3656, n3657*
9	*n3659, n3662*
9	*n3660, n3661*
17	*n3666, n3667*

All other isolates from independent plates.

Figure B.4

mod-5 enhancer screen outline. *mod-5* mutant animals are mutagenized and grown for two generations. The F2 generation is food-deprived for 30 minutes and transferred to the middle of a ring-shaped bacterial lawn. Animals are allowed to migrate for 30 minutes and animals that remain immobilized at the inner ring of the bacterial lawn are picked as candidate enhancers.

Figure B.4

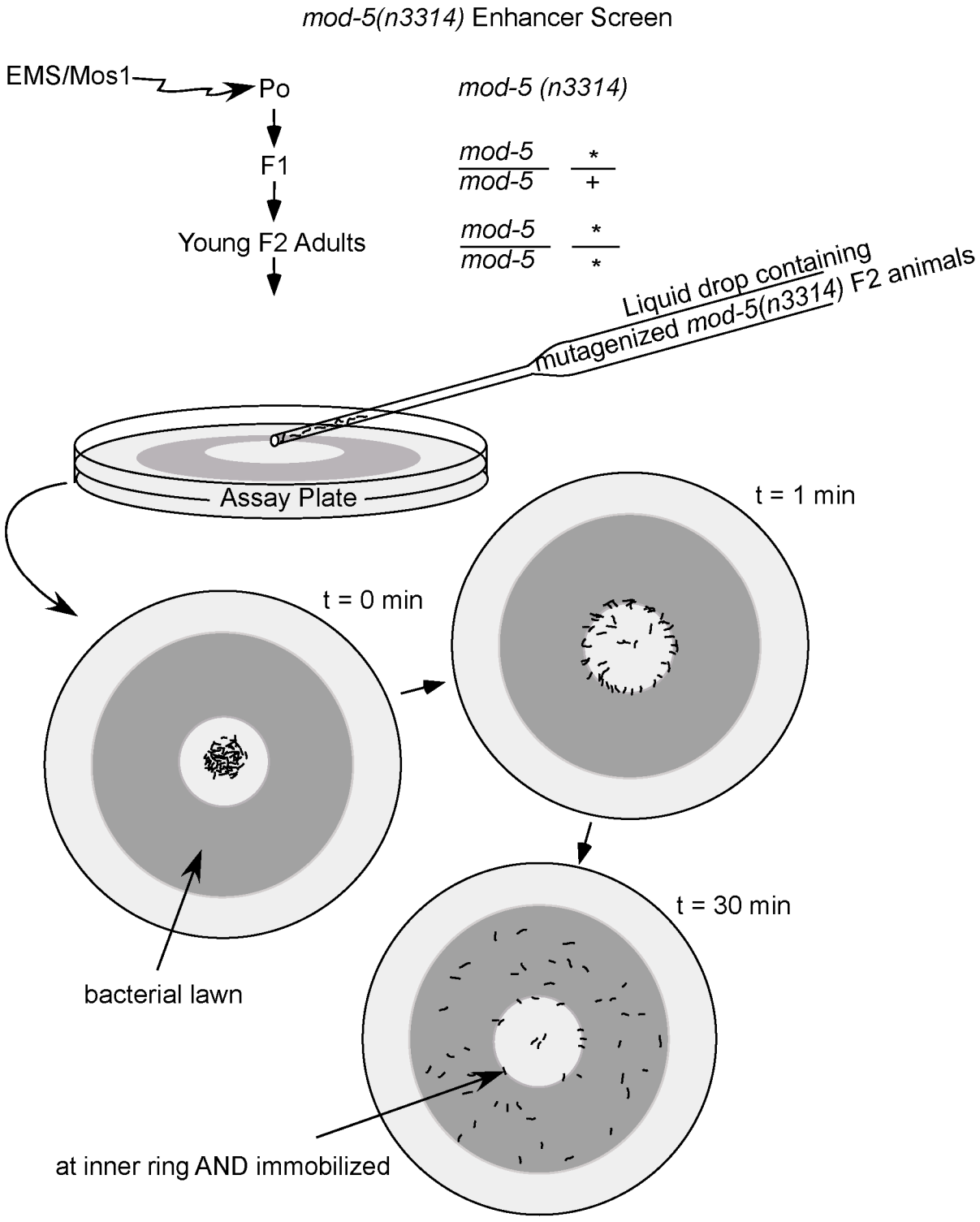

mod-5(n3314) Enhancer Screen
EMS/Mos1
Po
F1
Young F2 Adults
mod-5 (n3314)
mod-5 / mod-5 * / +
mod-5 / mod-5 * / *
Liquid drop containing mutagenized mod-5(n3314) F2 animals
Assay Plate
t = 0 min
t = 1 min
t = 30 min
bacterial lawn
at inner ring AND immobilized

Figure B.5

Summary of *mod-5* EMS enhancer screen isolate phenotypes with respect to locomotion off bacteria, locomotion on bacteria in the well-fed state, locomotion on bacteria in the well-fed state + methiothepin, growth rate, eggs retained per animal, reversals initiated by mechanical stimulation, and dependence of the food-dependent paralysis on the *mod-5* mutation. Dependence of *mod-5* mutation was tested by backcrossing strains to wild-type animas, re-isolating clones that exhibit food-dependent paralysis and genotyping strains at the *mod-5* locus. A designation of 'yes' signifies that all re-isolated clones contained the homozygous *mod-5(n3314)* mutation.

Figure B.5

Isolation name	genotype	well-fed locomotion			growth rate	eggs retained /animal	mechano-sensation	*mod-5* dependent
		no bacteria	bacteria	bacteria (methiothepin)				
starting	*n3314*	12.0	8.8	12.0	~63 hours	21.4	98%	n/a
i5-2	*n3314; n3924*	8.6	0.6	8.1	~66 hours	27.3	100%	yes
i16-1	*n3314; n3925*	10.6	0.1	12.8	63-68 hours	32.2	94%	n/d
i2-8-5	*n3314; n3948*	13.8	0.6	9.4	63-68 hours	30.5	96%	n/d
i3-2-3	*n3314; n4017*	12.0	0.0	8.8	n/d	n/d	8%	n/d
i3-2-4	*n3314; n4018*	10.4	0.0	5.8	~67 hours	34.1	44%	yes
i4-2-4	*n3314; n4019*	5.3	0.0	4.0	~68 hours	13.1	100%	yes
i4-13-2	*n3314; n4021*	9.2	0.0	4.6	63-68 hours	27.3	100%	n/d
i4-13-5	*n3314; n4022*	10.0	0.0	8.2	63-68 hours	27.5	86%	yes
		(body bends/20sec)			(late L4 to gravid adult)	(24 hours post L4)	(%responses)	

Complementation Groups

n3924, n3925/n4022, n3948, n4018, n4021

Preliminary Mapping

n3924 - chromosome IV
n4019 - chromosome I

Appendix C

Automated tracking data for mutants

Background

This appendix contains a collection of Mod Assay data of all mutants mentioned in previous chapters and many mutants that have not been mentioned in previous chapters. In our first table we present raw mutant data with wild-type controls gathered on identical days (Table C.1). This first table is less useful in comparing mutants to each other. This is due to the observation that data collected on the same day are more consistent than data collected on different days.

To compare the average locomotion rates for mutants assayed on different days, we used the following method. First wild-type control data from all days were be pooled to create a master wild-type dataset. A paired wild-type dataset is then created for each mutant, by pooling the wild-type control data for each day that specific mutant was assayed. To standardize data to the master control, we multiplied the average speed of each mutant in each condition (off-food, well-fed, food-deprived) by the ratio between the master wild-type control and paired wild-type control (value Master / value Paired) for each condition. Data calibrated by this method is presented in the second table (Table C.2). This method most successfully maintained the integrity of the relationships between mutants when compared to data collected completely in parallel. While this method generally preserves the relationships between mutants, quantification of small differences in locomotion rate ($P < .001$) between mutants should be done by comparing data collected strictly in parallel on many (>5) assay days. These data are graphically represented in the third table (Table C.3).

Early Mod Assays using the automated locomotion tracking system were performed on large 10 cm assay plates and animals were food-deprived in liquid S Basal medium. In these early experiments, the field of view used in the 10 cm assay was adjusted slightly each day, thus our data gathered from 10 cm plate should only be compared after using the above method for calibrating data using paired controls. Liquid food-deprivation was not the standard food-deprivation technique used in the original Mod Assay but was used in screens for Mod mutants. We find that liquid food deprivation does not significantly change the locomotion of wild-type animals of the few mutants we compared (Figure C.1). However, as not to introduce any unexpected

variables into the Mod Assay, we returned to food-depriving animals on plates. For a brief period, assays were also conducted on 5 cm plates with liquid food-deprivation. Data collected for the same mutant assayed under different conditions are kept separate. Assay conditions are specifically noted in all cases.

Figure C.1

Food-deprivation on plates and in liquid have the same effect on *C. elegans* locomotion on bacteria. Average speeds of wild-type, *tph-1(n4622)*, *tdc-1(n3420)*, *cat-2(n4547)*, and *npr-1(ky13)* animals tested in the off-bacteria, well-fed on bacteria, and food-deprived on bacteria conditions. Animals were food-deprived either on plates or in liquid S Basal media. The locomotion of *cat-2* mutants differ only in the two conditions that are not affected by food-deprivation. Data for solid and liquid food-deprivation experiments were not collected in parallel however the collection of large amounts of data usually leads to the large amount of reproducibility shown. The locomotion of *cat-2* mutants is highly variable in our assay for reasons described in Chapter 4. (N2 (wild type), n = 1797 no bacteria, 1466 well-fed, 1214 plate food-deprived tracks; N2 (wild type), n = 279, 221, 194 liquid food-deprived tracks; *tph-1(n4622)*, n = 274, 258, 214 solid, 189, 189, 186 liquid tracks; *tdc-1(n3420)*, n = 274, 258, 214 solid, 159, 131, 184 liquid tracks; *cat-2(n4547)*, n = 201, 321, 240 solid, 192, 133, 143 liquid; *npr-1(ky13)*, n = 475, 575, 412 solid, 132, 149, 106 liquid tracks)

Figure C.1

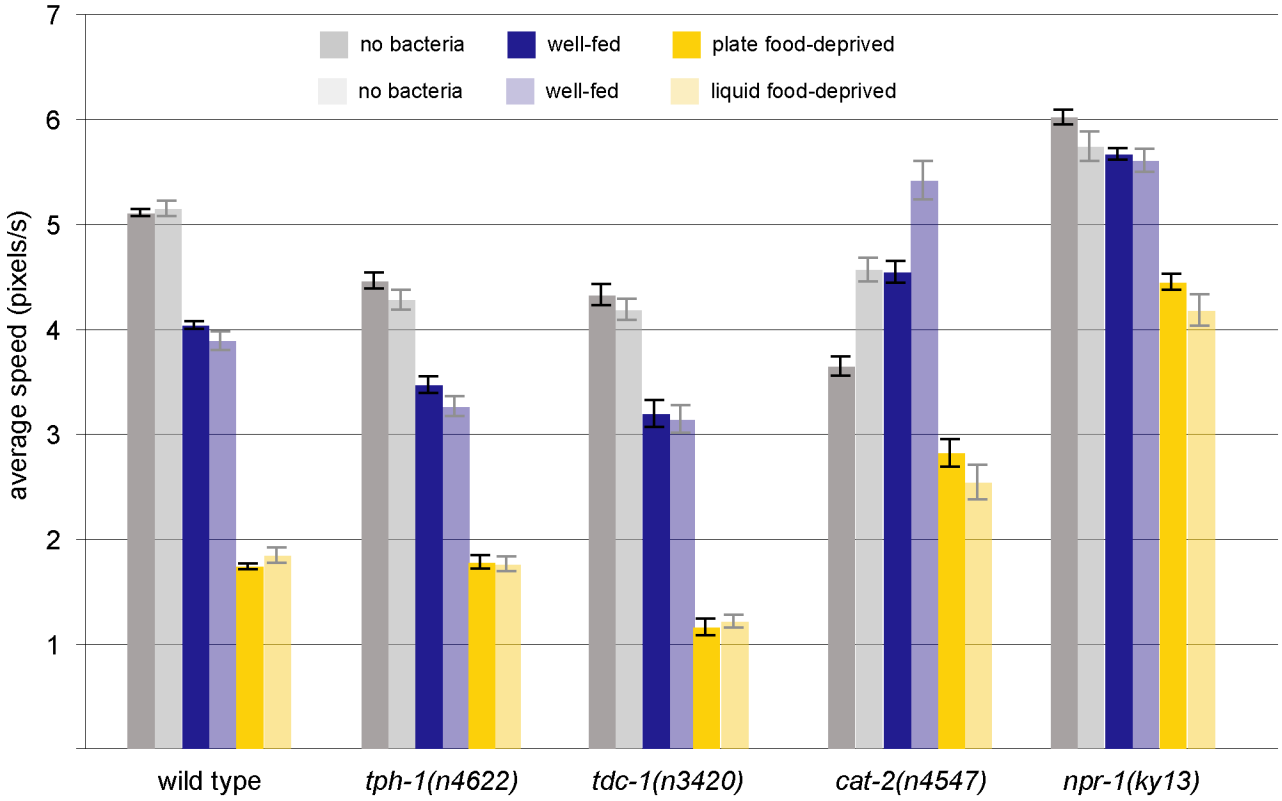

7
6
5
4
3
2
1
average speed (pixels/s)
no bacteria
well-fed
plate food-deprived
no bacteria
well-fed
liquid food-deprived
wild type
tph-1(n4622)
tdc-1(n3420)
cat-2(n4547)
npr-1(ky13)

Table C.1

Average speed data of animals tested in each condition as measured by the automated tracking system. Data for each mutant were collected on multiple days and pooled. Wild-type animals were assayed on identical days in parallel and pooled to make a paired wild-type control. A paired wild-type control is reported alongside each mutant. An asterisk in the 'single trial' column notes that the total data set comprises a data set collected on a single day and is only a very rough indication of that mutant phenotype. Food deprivation was performed in liquid S Basal medium and on plates. The food-deprivation method used is listed in the 'food deprivation' column. Experiments were performed on both 5 cm and 10 cm plates. The plate size is listed in the 'plate' column. The mean of all speed recordings for all animals in each assay condition are reported. n = the number of tracks collected in all experiments for that mutant or paired wild-type control.

Table C.1

Genotype/Strain	single trial	plate format	food deprivation	wild type									mutant								
				N2	stdev	n	N2	stdev	n	N2	stdev	n	MUT	stdev	n	MUT	stdev	n	MUT	stdev	n
age-1(hx546) II fer-15(b26) II		5 cm	plate	5.8465	1.3821	90	3.8299	1.5978	67	1.7525	1.0581	75	4.9157	1.2573	96	2.947	1.3618	55	0.8244	0.4385	45
bas-1(ad446) III ; cat-4(e1141) V		5 cm	plate	4.7509	1.1453	238	3.7377	1.181	168	1.8353	1.0572	151	3.4227	1.2895	289	4.3218	1.31	180	3.0089	1.5482	161
bas-1(ad446) III ; cat-4(e1141) V		5 cm	liquid	4.8724	1.1439	148	3.4683	1.3082	102	1.8573	1.0048	87	3.5956	1.4002	168	4.6222	1.1671	114	3.0655	1.6823	88
C25G6.5(ok594) X		5 cm	plate	5.272	1.1725	71	4.6935	1.1152	48	1.7382	0.8865	38	4.7817	1.3855	74	3.7664	1.3341	49	1.728	1.0409	36
C30F12.6(ok1381) I		5 cm	plate	5.272	1.1725	71	4.6935	1.1152	48	1.7382	0.8865	38	4.7694	1.3963	74	4.3886	1.5228	45	1.7931	0.9401	30
cat-2(e1112) II		5 cm	plate	4.775	1.3577	114	3.8189	1.368	83	1.5626	0.944	65	4.6962	1.4689	171	5.7989	1.608	123	2.664	2.0448	96
cat-2(e1112) II		10 cm	liquid	2.9339	0.6095	95	2.5274	0.6658	89	1.0409	0.4949	84	2.668	0.7668	156	3.3612	0.9635	156	2.8361	1.1173	119
cat-2(n4547) II		5 cm	plate	4.6226	1.2617	341	3.6651	1.2379	281	1.6527	0.9708	261	3.6408	1.3023	201	4.5408	1.8112	321	2.8165	2.0401	240
cat-2(n4547) II		5 cm	liquid	4.96	1.201	174	3.6579	1.3114	138	1.8322	1.0334	135	4.563	1.5491	192	5.4141	2.1367	133	2.5386	1.9937	143
cat-2(n4547) II		10 cm	liquid	3.0446	0.8876	234	2.6415	0.7779	205	1.192	0.5669	202	2.7695	1.0414	329	3.3481	1.1296	269	2.3317	1.1719	259
cat-2(n4547) II ; daf-2(e1370ts) III	*	10 cm	liquid	2.549	0.6729	44	2.0684	0.5664	27	0.9189	0.4399	34	1.7886	0.6659	44	2.596	1.1251	37	1.3958	1.1015	32
cat-2(n4547) II ; daf-28(sa191) V	*	10 cm	liquid	2.549	0.6729	44	2.0684	0.5664	27	0.9189	0.4399	34	1.9462	0.6815	41	3.0472	1.0126	32	1.3907	1.0048	32
cat-2(n4547) II ; flp-21(ok889) V		10 cm	liquid	2.3074	0.6742	78	2.0966	0.6681	58	1.0562	0.5005	44	2.0606	0.8405	80	3.1021	0.887	76	2.4745	1.2435	63
cat-2(n4547) II ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	plate	5.3965	1.186	100	4.0952	1.1666	99	1.7903	1	86	5.1565	1.7723	100	3.4402	2.5155	94	1.3753	1.1933	87
cat-2(n4547) II ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	liquid	5.3691	1.2092	103	4.0749	1.182	101	1.8037	1.0102	98	5.0127	1.8797	106	3.1311	2.4505	128	1.3922	1.1753	130
cat-2(n4547) II ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		10 cm	liquid	2.9977	0.6434	64	2.5726	0.6317	64	1.0386	0.5188	63	2.7409	0.8709	123	2.639	1.3986	101	1.1197	0.9528	80
cat-2(n4547) II ; mod-1(ok103) V		10 cm	liquid	2.7241	0.6951	54	2.4826	0.5826	39	1.1025	0.5526	47	2.0085	0.6273	66	2.4329	0.8745	57	1.0176	0.5955	51
cat-2(n4547) II ; mrp-1(pk89) X		5 cm	plate	6.2767	1.3958	97	4.6883	1.4752	77	2.2177	1.1961	54	5.0882	1.8776	86	5.593	1.9344	86	1.9913	1.7645	44
cat-2(n4547) II ; mrp-1(pk89) X		10 cm	liquid	2.5844	0.7015	89	2.1366	0.6584	71	1.0787	0.5031	78	2.2507	0.8091	132	2.605	1.0107	107	1.8841	1.2205	96
cat-2(n4547) II ; npr-1(ky13) X		5 cm	plate	5.1593	1.1313	102	3.7706	1.2806	70	1.7919	0.9835	62	4.9385	1.5271	101	5.3999	1.9575	96	3.9828	2.2383	62
cat-2(n4547) II ; npr-1(ky13) X		5 cm	liquid	5.1301	1.148	104	3.7599	1.2851	72	1.7909	0.9901	79	4.7495	1.7117	109	5.2464	2.0656	102	3.754	2.2979	68
cat-2(n4547) II ; npr-1(ky13) X		10 cm	liquid	3.1267	0.928	217	2.6922	0.8311	220	1.1155	0.5713	172	2.9341	1.246	338	3.4369	1.1563	269	3.3967	1.0741	255
cat-2(n4547) II ; tbh-1(n3722) X		5 cm	plate	4.2156	1.1709	138	3.6202	1.1952	130	1.4994	0.8958	126	3.2385	1.5735	195	2.6917	1.7852	121	0.9501	0.8248	89
cat-2(n4547) II tdc-1(n3420) II		5 cm	plate	5.0039	1.1752	179	3.571	1.2861	117	1.8381	0.9955	96	4.1768	1.4441	189	3.6562	1.908	134	1.205	1.0356	103
cat-2(n4547) II tdc-1(n3420) II		5 cm	liquid	4.8724	1.1439	148	3.4683	1.3082	102	1.8573	1.0048	87	4.2415	1.4087	169	3.6658	1.8258	120	1.3224	1.0869	132
cat-2(n4547) II tph-1(n4622) II		5 cm	plate	4.8279	1.1813	271	3.7309	1.2052	191	1.8272	1.0497	170	4.1945	1.4052	338	4.3804	1.7085	223	2.2756	1.8652	160
cat-2(n4547) II tph-1(n4622) II		5 cm	liquid	4.8724	1.1439	148	3.4683	1.3082	102	1.8573	1.0048	87	4.1226	1.4892	172	3.6386	1.9424	111	2.1943	1.805	102
cat-2(n4547) II tph-1(n4622) II		10 cm	liquid	2.7841	0.6838	100	2.4675	0.608	79	1.2043	0.5388	107	2.3971	0.8043	123	2.6735	0.9895	118	1.5483	1.0399	100
cat-2(n4547) II tph-1(n4622) II tdc-1(n3420) II		5 cm	plate	5.941	1.2526	124	4.7631	1.4498	107	1.9381	1.0108	64	6.5069	1.4519	137	5.4502	1.3393	120	1.8846	1.3063	60
CB4856 - Hawaiian wild-type strain		10 cm	liquid	2.6443	0.6619	102	2.2773	0.6201	103	1.0801	0.4776	126	3.394	0.9066	122	3.411	0.7958	103	2.5434	0.9709	121
che-1(p674) I		5 cm	plate	5.1597	1.3848	91	3.7345	1.3582	61	1.4404	0.8837	61	6.4402	1.809	79	5.4042	1.8741	100	2.3164	1.8256	34
che-3(e1124) I		5 cm	plate	5.1597	1.3848	91	3.7345	1.3582	61	1.4404	0.8837	61	2.5293	1.0931	49	0.9822	0.6185	48	1.0544	0.6772	23
che-3(e1124) I	*	10 cm	liquid	2.0488	0.627	44	1.8275	0.5192	36	0.8522	0.3227	23	1.1345	0.5029	38	0.9096	0.4507	17	0.7671	0.3915	16
che-3(e1124) I ; mrp-1(pk89) X	*	5 cm	plate	7.339	1.1487	29	4.3546	1.7102	32	2.2228	1.2321	24	3.5305	1.1553	15	1.2336	0.7693	15	0.9656	0.6692	19
che-3(n1512) I		5 cm	plate	5.1597	1.3848	91	3.7345	1.3582	61	1.4404	0.8837	61	3.977	1.4159	39	1.8857	1.1495	55	1.3997	0.8892	43
daf-1(m40ts) IV		5 cm	plate	6.1573	1.3396	101	4.368	1.8036	77	1.752	0.909	58	5.4361	1.6131	105	4.4921	1.7707	44	2.0922	1.3102	55
daf-11(m47ts) V		5 cm	plate	5.9481	1.3841	83	4.5782	1.7116	64	1.6093	1.0092	65	5.5097	1.2424	83	1.7542	1.1839	54	1.3014	0.7164	72
daf-11(m47ts) V ; mrp-1(pk89) X		5 cm	plate	5.7634	1.1571	97	3.8945	1.4713	81	1.7346	0.902	56	3.6991	1.1537	77	1.9486	0.8895	67	1.178	0.6273	63
daf-11(m47ts) V ; npr-1(ky13) X		5 cm	plate	5.7634	1.1571	97	3.8945	1.4713	81	1.7346	0.902	56	6.9475	1.9411	95	4.3008	2.2856	72	2.2929	1.3625	55
daf-12(m20) X		5 cm	plate	6.1382	1.4153	88	4.2215	1.8699	63	1.6011	0.9459	61	5.1278	1.3946	97	4.5326	1.368	81	1.3528	0.8234	55
daf-2(e1370ts) III		5 cm	plate	5.8396	1.2589	180	4.1891	1.6151	145	1.667	0.9633	121	3.4337	1.3322	174	1.8003	1.009	135	0.7454	0.5417	76
daf-2(e1370ts) III	*	10 cm	liquid	2.549	0.6729	44	2.0684	0.5664	27	0.9189	0.4399	34	2.2999	0.7673	34	1.3629	0.6272	33	0.5277	0.3679	21
daf-2(e1370ts) III ; mrp-1(pk89) X		5 cm	plate	5.8585	1.3035	129	4.748	1.4362	93	2.0146	1.1281	64	3.7104	1.4729	108	1.8111	1.2545	53	0.7982	0.5833	45
daf-2(e1370ts) III ; npr-1(ky13) X		5 cm	plate	5.8168	1.2422	226	4.3502	1.5137	174	1.8911	1.0437	120	6.559	1.632	188	6.17	1.6744	147	4.6418	1.9285	115
*daf-21(p673) V****		5 cm	plate	6.0224	1.3856	124	4.4817	1.6676	85	1.701	1.06	92	6.3762	1.1947	125	2.0496	1.3671	67	2.106	1.2226	96
daf-21(p673) V ; mrp-1(pk89) X		5 cm	plate	5.7634	1.1571	97	3.8945	1.4713	81	1.7346	0.902	56	5.1993	1.3449	98	1.8861	1.1931	102	1.3249	0.8618	52
*daf-21(p673) V ; npr-1(ky13) X ******		5 cm	plate	5.7634	1.1571	97	3.8945	1.4713	81	1.7346	0.902	56	5.639	1.6694	94	1.8388	1.1157	83	1.7129	0.8906	70
daf-28(sa191) V		10 cm	liquid	2.549	0.6729	44	2.0684	0.5664	27	0.9189	0.4399	34	2.5135	0.7631	45	2.4925	0.8468	32	0.739	0.4244	28
daf-3(e1376) X		5 cm	plate	6.0984	1.2605	94	4.2024	1.7115	71	1.7139	0.9831	65	5.4798	1.156	98	3.662	1.4113	66	1.5068	0.9066	46
daf-5(e1386) II		5 cm	plate	6.1382	1.4153	88	4.2215	1.8699	63	1.6011	0.9459	61	4.7484	1.1767	85	3.3297	1.2535	58	1.217	0.7393	48

Locomotion off Bacteria (pixels/second) | Well-fed Locomotion on Bacteria (pixels/second) | Food-Deprived Locomotion on Bacteria (pixels/second)

*****strain may be incorrect*

Table C.1 - continued

Genotype/Strain	single trial	plate format	food deprivation	wild type									mutant								
				N2	stdev	n	N2	stdev	n	N2	stdev	n	MUT	stdev	n	MUT	stdev	n	MUT	stdev	n
daf-7(e1372ts) III		5 cm	plate	5.8336	1.2692	105	4.5655	1.4311	88	1.7522	1.0128	66	3.435	2.0417	74	4.6574	1.9359	61	2.3287	1.5667	69
daf-7(e1372ts) III	*	10 cm	liquid	2.549	0.6729	44	2.0684	0.5664	27	0.9189	0.4399	34	3.1384	0.8838	30	3.0417	1.1278	30	1.0178	0.593	12
daf-8(e1393ts) I		5 cm	plate	5.847	1.3121	98	4.0172	1.7347	81	1.6938	0.9436	62	5.2043	1.5544	99	3.762	1.9002	43	2.0485	1.1498	68
dat-1(ok157) III		5 cm	plate	4.8545	1.1962	45	4.6465	1.1669	38	1.8032	0.8868	31	5.2107	1.536	51	4.2763	1.5	30	1.8915	1.3913	18
dop-1(vs100) X		5 cm	plate	4.8545	1.1962	45	4.6465	1.1669	38	1.8032	0.8868	31	4.7799	1.2849	55	4.2113	1.5453	36	1.3537	0.8714	21
dop-2(vs105) V		5 cm	plate	4.8545	1.1962	45	4.6465	1.1669	38	1.8032	0.8868	31	4.2054	1.1273	55	3.5303	1.039	38	1.7014	0.8408	25
dop-2(vs105) V ; dop-3(vs106) X		5 cm	plate	4.8273	1.374	50	4.5938	1.1233	65	1.6616	0.9639	45	4.7188	1.2387	41	3.3613	1.9709	65	1.9694	1.5014	40
dop-3(vs106) X		5 cm	plate	4.8545	1.1962	45	4.6465	1.1669	38	1.8032	0.8868	31	5.9555	1.4339	88	6.0527	1.9072	76	1.8362	1.6273	28
dop-4(ok1321) X		5 cm	plate	4.952	1.286	50	4.3107	1.2817	33	2.0938	1.2025	36	4.4551	1.4486	56	4.4292	1.1792	50	1.9533	0.9699	29
F14D12.6(ok371) X		5 cm	plate	4.8545	1.1962	45	4.6465	1.1669	38	1.8032	0.8868	31	4.5003	1.242	52	3.3401	1.1989	46	1.275	0.7408	26
F41E7.3(tm1497) X	*	5 cm	plate	4.4069	1.2296	28	3.5861	1.0836	13	1.3084	0.7446	20	4.5145	1.1936	16	2.914	1.4107	14	0.9101	0.4711	13
flp-11(n4765) X		5 cm	plate	6.1915	1.1324	56	4.1614	1.6424	47	1.5149	0.8648	43	4.1696	1.3418	49	3.1814	1.2912	36	1.5522	0.9049	32
flp-18(n4766) X		10 cm	liquid	3.0255	0.5874	99	2.691	0.6807	63	1.1478	0.5336	58	2.2617	0.817	83	2.1429	0.8336	59	0.9147	0.4478	63
flp-18(n4766) X		5 cm	plate	6.2928	1.345	96	4.6651	1.6181	59	1.7697	1.0842	70	6.0914	1.2769	114	4.5381	1.7398	60	1.7345	0.9173	52
flp-18(n4766) X flp-11(n4765) X	*	5 cm	plate	6.1652	1.0781	39	3.731	1.5986	23	1.9502	0.9173	22	5.2353	1.5073	48	4.3919	1.2862	24	1.3152	0.7993	16
flp-21(ok889) V		5 cm	plate	6.2631	1.2274	111	4.4657	1.6773	85	1.5815	0.9488	86	6.3915	1.5752	127	5.4042	1.7228	114	2.7654	1.2987	76
flp-21(ok889) V		10 cm	liquid	2.5566	0.7763	63	2.2195	0.6299	41	0.9207	0.4833	54	2.4392	0.8193	63	2.5301	0.7754	56	1.0236	0.4476	49
flp-21(ok889) V ; flp-11(n4765) X		5 cm	plate	6.1793	1.1074	95	4.0117	1.64	70	1.6921	0.9119	65	5.9018	1.4923	161	4.4631	1.5681	100	2.4244	1.1998	87
flp-21(ok889) V ; flp-18(n4766) X		5 cm	plate	6.1793	1.1074	95	4.0117	1.64	70	1.6921	0.9119	65	5.8171	1.4011	115	5.0538	1.6606	98	1.8858	1.0892	48
goa-1(n1134) I		5 cm	plate	5.4692	1.5703	86	3.6519	1.3601	68	1.36	0.7846	65	6.184	1.4255	81	5.0208	2.0171	96	1.9773	1.6563	70
goa-1(n1134) I		10 cm	liquid	2.7241	0.6951	54	2.4826	0.5826	39	1.1025	0.5526	47	3.1956	0.8196	41	2.1904	1.3129	37	1.2099	1.0502	51
irk-1(n4895) X		5 cm	plate	4.9881	1.1907	96	4.4611	1.1807	67	1.6252	0.9141	54	5.3761	1.419	103	4.6669	1.7788	63	1.6508	1.0531	54
irk-2(n4896) X		5 cm	plate	5.482	1.5024	80	4.5766	1.5195	57	1.5553	1.0037	59	4.5654	1.2221	95	2.8146	1.5781	53	1.1037	0.6899	64
lin-15AB(n765ts) X ; nEx1242/3 (flp-11 overexp)		10 cm	liquid	3.1246	0.6368	122	2.7766	0.6823	97	1.152	0.5278	73	3.0522	0.8603	106	1.2813	0.9178	77	0.6628	0.4951	89
lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	plate	5.3965	1.186	100	4.0952	1.1666	99	1.7903	1	86	5.0234	1.2339	98	3.3628	1.7949	75	1.2917	0.8857	65
lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	liquid	5.3691	1.2092	103	4.0749	1.182	101	1.8037	1.0102	98	5.0097	1.2362	98	3.3275	1.7881	79	1.329	0.9129	99
lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		10 cm	liquid	2.9977	0.6434	64	2.5726	0.6317	64	1.0386	0.5188	63	2.6825	0.8078	94	2.1619	0.8873	73	0.7179	0.4649	59
lin-15AB(n765ts) X ; nEx1277 (flp-18 overexp)		10 cm	liquid	3.1365	0.6682	81	2.8204	0.6624	83	1.1327	0.5219	64	3.093	0.9521	95	2.6691	0.8806	99	1.2334	0.6848	76
mgDf50 I		5 cm	plate	5.5804	1.3451	87	3.962	1.771	80	1.6921	0.9767	48	3.4673	1.207	77	2.7099	0.9439	56	1.1773	0.7681	57
mod-1(n3034) V		5 cm	plate	4.616	1.2057	99	3.8817	1.3519	97	1.9524	1.0456	76	4.4982	1.36	143	4.5172	1.1972	97	1.631	1.0196	84
mod-1(n3034) V ; n4954		5 cm	plate	4.616	1.2057	99	3.8817	1.3519	97	1.9524	1.0456	76	4.7593	1.5508	103	5.1167	1.1841	98	3.2054	1.3787	99
mod-1(n3034) V ; n4954		10 cm	liquid	3.1707	0.6796	128	2.7177	0.6278	91	1.1342	0.5774	91	3.207	0.8623	140	2.8531	0.778	92	1.5648	0.5845	79
mod-1(nr2043) V		5 cm	plate	4.616	1.2057	99	3.8817	1.3519	97	1.9524	1.0456	76	4.4569	1.4335	107	4.1748	1.2273	109	1.7251	1.0411	86
mod-1(ok103) V		5 cm	plate	4.8173	1.252	232	3.7598	1.3786	226	1.7798	1.0139	146	4.5299	1.3702	212	4.1806	1.3084	189	1.6078	1.0104	161
mod-1(ok103) V		5 cm	liquid	5.4305	1.1144	86	3.9671	1.3491	89	1.7689	0.9557	48	5.0723	1.816	52	4.3608	1.1353	52	1.5819	1.013	58
mod-1(ok103) V		10 cm	liquid	2.5566	0.7763	63	2.2195	0.6299	41	0.9207	0.4833	54	2.2723	0.7047	42	1.6212	0.7404	56	0.6284	0.3711	39
mod-1(ok103) V ; lin-15AB(n765ts) X ; nEx1252		5 cm	liquid	5.43	1.2436	79	3.9317	1.2016	93	1.8017	1.0291	81	4.4379	1.2772	82	2.5684	1.7314	76	1.2328	0.8097	114
mod-1(ok103) V ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	plate	5.4641	1.21	77	3.954	1.1861	91	1.7813	1.0196	69	4.4836	1.2805	103	2.6369	1.7542	61	1.1826	0.7426	98
mod-1(ok103) V ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		10 cm	liquid	2.9977	0.6434	64	2.5726	0.6317	64	1.0386	0.5188	63	2.4491	0.7628	86	1.6668	0.9585	66	0.7529	0.4951	70
mod-5(n3314) I		5 cm	plate	5.4548	1.2684	274	3.8496	1.4065	231	1.6045	0.9156	160	4.4303	1.544	226	2.1772	1.2652	174	0.7588	0.4922	153
mod-5(n3314) I		5 cm	liquid	5.4305	1.1144	86	3.9671	1.3491	89	1.7689	0.9557	48	3.8659	1.3978	58	2.0882	1.2273	64	1.1625	0.9401	80
mod-5(n3314) I		10 cm	liquid	2.5566	0.7763	63	2.2195	0.6299	41	0.9207	0.4833	54	2.4365	0.778	61	1.1195	0.5711	55	0.5465	0.4172	30
mod-5(n3314) I ; cat-2(e1112) II		5 cm	plate	5.1597	1.3848	91	3.7345	1.3582	61	1.4404	0.8837	61	4.5859	1.6239	117	3.8375	2.4596	83	0.9242	0.6407	51
mod-5(n3314) I ; cat-2(n4547) II tph-1(n4622) II tdc-1(n3420) II	*	5 cm	plate	6.1652	1.0781	39	3.731	1.5986	23	1.9502	0.9173	22	3.1706	1.7919	21	3.997	2.025	12	1.115	0.7526	14
mod-5(n3314) I ; daf-1(m40ts) IV		5 cm	plate	5.7099	1.2714	94	4.9846	1.2447	69	2.1412	1.16	47	5.6047	1.1755	74	2.7094	1.5987	54	0.8743	0.5321	41
mod-5(n3314) I ; daf-11(m87) V		5 cm	plate	5.7634	1.1571	97	3.8945	1.4713	81	1.7346	0.902	56	4.1984	1.5639	40	1.6199	1.0969	39	1.2461	0.9473	31
mod-5(n3314) I ; daf-2(e1370ts) III	*	10 cm	liquid	2.549	0.6729	44	2.0684	0.5664	27	0.9189	0.4399	34	2.542	0.8206	42	0.9626	0.4759	23	0.8579	0.5818	23
mod-5(n3314) I ; daf-7(e1372ts) III		5 cm	plate	5.7099	1.2714	94	4.9846	1.2447	69	2.1412	1.16	47	4.9335	1.9659	65	1.6838	1.4103	36	1.0944	0.9824	39
mod-5(n3314) I ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	plate	5.3965	1.186	100	4.0952	1.1666	99	1.7903	1	86	2.791	1.1937	86	1.0325	0.6891	79	0.8723	0.648	65
mod-5(n3314) I ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	liquid	5.3691	1.2092	103	4.0749	1.182	101	1.8037	1.0102	98	2.7521	1.1968	102	1.1142	0.773	120	1.1181	0.894	112

Locomotion off Bacteria (pixels/second) | Well-fed Locomotion on Bacteria (pixels/second) | Food-Deprived Locomotion on Bacteria (pixels/second)

Table C.1 - continued

Genotype/Strain	single trial	plate format	food deprivation	wild type									mutant								
				N2	stdev	n	N2	stdev	n	N2	stdev	n	MUT	stdev	n	MUT	stdev	n	MUT	stdev	n
mod-5(n3314) I ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		10 cm	liquid	2.9977	0.6434	64	2.5726	0.6317	64	1.0386	0.5188	63	1.7679	0.6215	63	0.7452	0.5106	67	0.6903	0.458	50
mod-5(n3314) I ; npr-1(ky13) X		10 cm	liquid	2.7025	0.6703	81	2.2553	0.6665	66	1.1962	0.488	95	3.0813	0.7432	67	2.7162	0.7733	61	1.6807	0.7461	79
mod-5(n3314) I ; tdc-1(n3420) II		5 cm	plate	6.5463	1.3951	80	4.5661	1.5813	58	2.2795	1.2041	38	3.1327	1.4252	42	1.2694	0.733	30	0.7812	0.6378	19
mod-5(n3314) I ; tph-1(n4622) II		5 cm	plate	5.4844	1.1783	117	4.0651	1.3417	106	1.8434	1.0283	58	5.1823	0.9567	98	4.0551	1.1025	97	2.1849	1.0362	79
mod-5(n3314) I ; tph-1(n4622) II		5 cm	liquid	5.4305	1.1144	86	3.9671	1.3491	89	1.7689	0.9557	48	5.2605	0.9697	82	4.0111	1.2429	85	2.2797	1.0715	66
mod-5(n3314) I ; tph-1(n4622) II		10 cm	liquid	2.6261	0.6232	59	2.1762	0.6469	54	1.1524	0.448	75	2.6305	0.6324	47	2.4396	0.641	42	1.2304	0.5254	90
mod-5(n3314) I ; tph-1(n4622) II tdc-1(n3420) II	*	5 cm	plate	6.1652	1.0781	39	3.731	1.5986	23	1.9502	0.9173	22	4.9745	1.9562	39	2.5947	1.5406	6	2.0577	0.8766	19
mod-5(n3314) I daf-8(e1393ts) I		5 cm	plate	5.7099	1.2714	94	4.9846	1.2447	69	2.1412	1.16	47	5.4675	1.3913	68	3.2164	1.7828	55	0.8127	0.6394	52
mod-6(n3076) I		5 cm	plate	5.0904	1.2	135	4.1873	1.3191	127	1.6539	0.9164	79	5.0774	1.3773	102	3.9809	1.6253	112	2.7174	1.4512	68
mod-6(n3076) I		5 cm	liquid	5.4305	1.1144	86	3.9671	1.3491	89	1.7689	0.9557	48	5.3428	1.3687	68	3.7631	1.6424	69	2.8771	1.4528	52
mod-6(n3076) I		10 cm	liquid	2.476	0.7467	51	2.1953	0.5135	51	0.9697	0.4854	46	2.4715	0.8264	50	2.2677	0.7498	45	1.0793	0.5738	35
mrp-1(pk89) X		5 cm	plate	5.3432	1.4046	213	3.798	1.4624	188	1.6825	0.9271	143	3.7953	1.3508	175	2.448	1.2146	178	1.1602	0.6804	152
mrp-1(pk89) X		10 cm	liquid	2.5566	0.7763	63	2.2195	0.6299	41	0.9207	0.4833	54	1.9557	0.7214	68	1.3826	0.7208	49	0.4522	0.2926	37
mrp-1(pk89) X npr-1(ky13) X		5 cm	plate	4.4696	1.1818	60	3.6739	1.2995	63	1.6487	0.9546	44	5.4142	1.2904	69	4.9851	1.0922	62	4.0874	1.4179	60
N2		5 cm	plate	5.103	1.4121	1797	4.0319	1.4111	1466	1.7367	1.013	1214	5.103	1.4121	1797	4.0319	1.4111	1466	1.7367	1.013	1214
N2		5 cm	liquid	5.1443	1.2134	279	3.8863	1.3073	221	1.8422	1.0176	194	5.1443	1.2134	279	3.8863	1.3073	221	1.8422	1.0176	194
N2		10 cm	liquid	2.8735	0.7697	1194	2.5291	0.7377	1027	1.1076	0.5372	944	2.8735	0.7697	1194	2.5291	0.7377	1027	1.1076	0.5372	944
n4954		5 cm	plate	4.616	1.2057	99	3.8817	1.3519	97	1.9524	1.0456	76	5.1076	1.3045	126	4.9142	1.216	102	2.8022	1.2619	81
npr-1(ky13) X		5 cm	plate	5.0302	1.335	517	3.8335	1.3372	388	1.7058	1.0083	324	6.015	1.5424	475	5.6652	1.3431	575	4.4459	1.582	412
npr-1(ky13) X		5 cm	liquid	5.1936	1.1491	136	3.7431	1.285	95	1.7904	0.9889	95	5.7377	1.612	132	5.6045	1.3324	149	4.1751	1.5368	106
npr-1(ky13) X		10 cm	liquid	2.9956	0.982	270	2.5363	0.861	254	1.1013	0.566	188	2.8909	1.0097	185	3.2891	1.0138	213	3.075	1.0077	256
npr-1(ky13) X lin-15AB(n765ts) X ; flp-21 overexp		10 cm	liquid	3.3811	0.7574	120	2.6768	0.8135	115	1.1618	0.5028	71	3.9628	0.8079	71	3.4178	1.0223	52	3.458	0.9125	78
npr-1(ky13) X lin-15AB(n765ts) X ; nEx1242/3 (flp-11 overexp)		10 cm	liquid	3.3811	0.7574	120	2.6768	0.8135	115	1.1618	0.5028	71	3.9816	0.9206	64	3.9547	0.9405	90	3.1813	1.1654	63
npr-1(ky13) X lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)	*	5 cm	plate	5.4816	1.1104	32	3.5766	1.2824	23	1.7788	0.9671	15	4.1929	1.0955	10	1.7354	0.8478	11	0.9481	0.6571	17
npr-1(ky13) X lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)	*	5 cm	liquid	5.4789	1.1105	32	3.5748	1.2741	23	1.787	0.9819	16	4.1804	1.0988	10	1.598	0.7099	13	1.1849	0.8948	34
npr-1(ky13) X lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		10 cm	liquid	2.9739	0.6614	120	2.77	0.6816	108	1.2181	0.5975	133	2.6686	0.8485	105	2.2834	1.137	74	0.8154	0.6464	134
npr-1(ky13) X lin-15AB(n765ts) X ; nEx1277 (flp-18 overexp)		10 cm	liquid	3.1565	0.7548	79	2.7604	0.7359	74	1.1308	0.4569	45	3.6421	0.934	25	3.9611	0.827	47	3.4304	1.0907	68
npr-1(ky13) X osm-1(p808) X	*	5 cm	plate	4.2871	1.0792	32	3.4935	1.3854	22	1.2975	0.7767	18	4.0173	1.292	26	3.4462	1.4562	26	1.4567	0.7358	13
npr-1(ok1447) X		5 cm	plate	5.4473	1.4606	93	3.9498	1.3519	52	1.579	0.9377	54	5.3401	1.5171	82	4.7338	1.3747	74	4.0314	1.422	73
npr-1(ok1447) X	*	10 cm	liquid	2.3817	0.5553	23	2.3515	0.609	32	0.984	0.4813	25	3.049	0.9013	37	3.5413	0.7537	37	2.7559	0.8688	34
npr-2(ok419) IV		5 cm	plate	4.6325	1.2609	182	4.0188	1.1847	122	1.7574	1.078	107	2.3476	1.5326	151	0.8045	0.6624	95	0.7798	0.6402	52
npr-2(ok419) IV ; npr-1(ky13) X		5 cm	plate	4.402	1.2113	111	3.7481	1.1008	74	1.7661	1.1545	69	2.7128	1.5705	122	1.8895	1.7605	84	1.5799	1.3853	67
npr-3(tm1583) IV	*	5 cm	plate	4.4069	1.2296	28	3.5861	1.0836	13	1.3084	0.7446	20	3.9124	1.0496	21	2.6405	1.198	17	0.9858	0.5343	17
osm-1(p808) X	*	5 cm	plate	4.2871	1.0792	32	3.4935	1.3854	22	1.2975	0.7767	18	2.5054	1.0021	32	2.0215	1.1787	27	1.0487	0.3953	2
osm-1(p808) X		10 cm	liquid	2.8092	0.6509	43	2.6795	0.6151	56	0.9869	0.4843	49	2.1879	0.935	47	1.2286	0.858	55	0.9001	0.5748	63
osm-6(p811) V		10 cm	liquid	2.8092	0.6509	43	2.6795	0.6151	56	0.9869	0.4843	49	1.7489	0.7149	56	1.0114	0.7215	53	0.8911	0.62	62
ser-1(ok345) X		5 cm	plate	4.952	1.286	50	4.3107	1.2817	33	2.0938	1.2025	36	5.3498	1.2744	50	4.5625	1.3495	50	2.187	1.2527	18
ser-2(pk1357) X		5 cm	plate	4.8545	1.1962	45	4.6465	1.1669	38	1.8032	0.8868	31	5.3132	1.2472	60	4.2004	1.1856	43	1.6989	1.0592	34
ser-3(ad1774) I		5 cm	plate	4.8545	1.1962	45	4.6465	1.1669	38	1.8032	0.8868	31	4.5013	1.5384	58	3.9342	1.2712	43	1.5772	0.9739	14
ser-4(ok512) III		10 cm	liquid	3.0636	0.6698	74	2.862	0.6636	53	1.1299	0.6044	72	3.1616	0.7358	50	3.0854	0.9283	50	1.1417	0.7097	68
ser-4(ok512) III (2x)		5 cm	plate	4.7818	1.3117	47	3.3043	1.3598	40	1.3811	0.8633	27	5.714	1.6579	130	4.9394	1.6076	85	1.9934	1.253	77
ser-7(n4542) X		5 cm	plate	4.8545	1.1962	45	4.6465	1.1669	38	1.8032	0.8868	31	5.0431	1.3484	62	4.0351	1.3273	44	0.9652	0.5487	19
T02E9.3(ok568) V		5 cm	plate	4.952	1.286	50	4.3107	1.2817	33	2.0938	1.2025	36	4.8726	1.243	56	4.3044	1.191	38	1.8696	1.2586	25
T21F2.1(n4330) X		5 cm	plate	4.952	1.286	50	4.3107	1.2817	33	2.0938	1.2025	36	4.0725	1.1947	52	3.9074	1.0487	50	2.0193	1.2102	31
T24D87(del) V		5 cm	plate	4.952	1.286	50	4.3107	1.2817	33	2.0938	1.2025	36	4.8623	1.2915	58	3.9928	1.4406	42	1.3778	1.0438	29
tax-2(p671) I		5 cm	plate	5.9183	1.2403	87	4.1779	1.5446	64	1.6768	0.9882	58	4.6997	1.4688	92	1.825	1.2284	55	0.9728	0.6857	46
tax-2(p671) I		10 cm	liquid	2.8092	0.6509	43	2.6795	0.6151	56	0.9869	0.4843	49	2.6344	0.7661	53	1.5579	0.9446	45	0.8358	0.6158	65
tax-2(p671) I ; npr-1(ky13) X		5 cm	plate	5.9183	1.2403	87	4.1779	1.5446	64	1.6768	0.9882	58	5.3921	1.7167	97	2.9128	1.9168	91	1.4089	1.1345	38
tax-2(p671) I ; npr-1(ky13) X	*	10 cm	liquid	3.1261	0.5465	40	2.5615	0.7486	35	1.2838	0.5386	18	3.0051	0.8758	29	1.9709	1.1547	32	1.5304	1.154	26
tax-4(p678) III		5 cm	plate	5.4523	1.4663	84	3.9903	1.5209	60	1.4387	0.8284	63	5.3635	1.6329	112	1.7832	1.1079	67	1.4877	1.241	47

Locomotion off Bacteria (pixels/second) | Well-fed Locomotion on Bacteria (pixels/second) | Food-Deprived Locomotion on Bacteria (pixels/second)

Table C.1 - continued

Genotype/Strain	single trial	plate format	food deprivation	wild type									mutant								
				N2	stdev	n	N2	stdev	n	N2	stdev	n	MUT	stdev	n	MUT	stdev	n	MUT	stdev	n
tax-4(p678) III		10 cm	liquid	2.8092	0.6509	43	2.6795	0.6151	56	0.9869	0.4843	49	2.9977	0.8553	65	1.8074	0.9632	57	0.9758	0.6507	56
tax-4(p678) III ; npr-1(ky13) X		5 cm	plate	5.4523	1.4663	84	3.9903	1.5209	60	1.4387	0.8284	63	5.0908	1.5266	88	2.6166	1.4895	65	1.2454	0.9442	51
tax-4(p678) III ; npr-1(ky13) X	*	10 cm	liquid	3.1261	0.5465	40	2.5615	0.7486	35	1.2838	0.5386	18	3.8658	1.0438	76	2.2	1.1385	47	1.608	1.1826	23
tax-5(p672) V	*	10 cm	liquid	2.574	0.6148	34	2.5375	0.6504	22	1.2118	0.5534	21	2.9692	1.028	64	2.9322	1.129	52	2.1026	1.1269	32
tbh-1(n3722) X		5 cm	plate	4.868	1.4822	322	3.7175	1.3082	260	1.5121	0.8983	244	4.4678	1.5486	286	3.8086	1.4025	261	1.5156	1.0565	215
tdc-1(n3420) II		5 cm	plate	4.9956	1.1695	188	3.8617	1.2774	140	1.8125	0.9969	115	4.3232	1.3118	173	3.1905	1.4609	133	1.1609	0.8198	110
tdc-1(n3420) II		5 cm	liquid	4.867	1.1366	158	3.5956	1.3007	126	1.8283	1.008	115	4.1825	1.2923	159	3.1399	1.475	131	1.2158	0.8158	184
tdc-1(n3420) II		10 cm	liquid	3.3873	0.7628	227	2.7981	0.7447	200	1.232	0.5957	182	3.189	0.789	231	2.6595	0.9222	162	0.8414	0.484	162
tdc-1(n3420) II ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	plate	5.3965	1.186	100	4.0952	1.1666	99	1.7903	1	86	3.8637	1.2427	118	1.6435	1.13	73	0.9002	0.5361	53
tdc-1(n3420) II ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	liquid	5.3691	1.2092	103	4.0749	1.182	101	1.8037	1.0102	98	3.8054	1.2598	124	1.5976	1.0896	104	1.1774	0.8427	132
tdc-1(n3420) II ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		10 cm	liquid	2.9977	0.6434	64	2.5726	0.6317	64	1.0386	0.5188	63	2.0377	0.7155	67	1.1976	0.8415	83	0.7227	0.5429	68
tdc-1(n3420) II ; npr-1(ky13) X		5 cm	plate	5.1593	1.1313	102	3.7706	1.2806	70	1.7919	0.9835	62	6.5448	1.3002	97	5.4653	1.4403	89	4.1477	1.6652	85
tdc-1(n3420) II ; npr-1(ky13) X		5 cm	liquid	5.1301	1.148	104	3.7599	1.2851	72	1.7909	0.9901	79	6.5389	1.2912	99	5.4552	1.445	90	4.0961	1.6857	90
tdc-1(n3420) II ; npr-1(ky13) X		10 cm	liquid	3.7102	0.7378	125	3.0134	0.761	124	1.2966	0.5992	88	4.2106	0.7803	88	4.2031	0.9205	140	3.5418	0.8785	136
tph-1(mg280) II		5 cm	plate	4.4823	1.2116	110	3.5872	1.2494	107	1.4716	0.8885	86	4.4395	1.1299	162	3.7817	1.0208	109	1.9659	0.9924	108
tph-1(n4622) II		5 cm	plate	4.9133	1.1738	277	3.7608	1.2641	249	1.7252	0.9602	185	4.4578	1.289	274	3.465	1.2839	258	1.7788	0.931	214
tph-1(n4622) II		5 cm	liquid	4.9529	1.1497	212	3.7115	1.3284	192	1.8154	0.9964	147	4.2751	1.3038	189	3.2614	1.3005	189	1.7587	0.9462	186
tph-1(n4622) II		10 cm	liquid	2.9306	0.9686	219	2.5701	0.8	199	1.1163	0.5686	162	2.5169	0.9302	221	2.2855	0.7706	198	1.057	0.527	150
tph-1(n4622) II ; bas-1(ad446) III ; cat-4(e1141) V		5 cm	plate	4.5815	1.1452	92	3.9192	1.0722	74	1.8167	1.0998	74	4.0979	1.2298	142	3.9146	1.3331	93	2.4048	1.5707	98
tph-1(n4622) II ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	plate	5.3965	1.186	100	4.0952	1.1666	99	1.7903	1	86	4.6464	1.3261	100	2.9138	1.6175	74	1.5274	0.806	78
tph-1(n4622) II ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	liquid	5.3691	1.2092	103	4.0749	1.182	101	1.8037	1.0102	98	4.5997	1.3427	103	2.8742	1.6179	82	1.5557	0.89	126
tph-1(n4622) II ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		10 cm	liquid	2.9977	0.6434	64	2.5726	0.6317	64	1.0386	0.5188	63	2.9984	0.7647	93	2.2048	0.8471	74	0.9619	0.5584	61
tph-1(n4622) II ; mod-1(ok103) V	*	10 cm	liquid	2.0488	0.627	44	1.8275	0.5192	36	0.8522	0.3227	23	1.7423	0.5454	42	1.9022	0.5703	30	0.8612	0.3205	19
tph-1(n4622) II ; mrp-1(pk89) X		10 cm	liquid	2.4397	0.7224	133	2.0405	0.6348	107	1.03	0.4793	101	2.3769	0.6839	102	1.7141	0.7787	120	0.7157	0.4384	104
tph-1(n4622) II ; npr-1(ky13) X		5 cm	plate	5.1593	1.1313	102	3.7706	1.2806	70	1.7919	0.9835	62	5.3374	1.6065	116	4.8152	1.626	103	4.3548	1.4607	97
tph-1(n4622) II ; npr-1(ky13) X		5 cm	liquid	5.1301	1.148	104	3.7599	1.2851	72	1.7909	0.9901	79	5.3067	1.6202	118	4.7998	1.6352	105	4.3273	1.4731	100
tph-1(n4622) II ; npr-1(ky13) X		10 cm	liquid	3.0314	1.0239	144	2.5799	0.8508	155	1.1179	0.5933	112	3.0214	1.2069	145	3.2664	0.9912	131	3.0226	1.0036	160
tph-1(n4622) II tdc-1(n3420) II		5 cm	plate	5.0039	1.1752	179	3.571	1.2861	117	1.8381	0.9955	96	4.7559	1.2092	157	3.9067	1.3175	124	2.0528	0.924	104
tph-1(n4622) II tdc-1(n3420) II		5 cm	liquid	4.8724	1.1439	148	3.4683	1.3082	102	1.8573	1.0048	87	4.5623	1.2706	131	3.647	1.3782	101	1.9814	0.9384	99
tyra-3(ok325) X		5 cm	plate	4.8545	1.1962	45	4.8465	1.1669	38	1.8032	0.8868	31	5.1146	1.3917	72	4.2989	1.0676	41	1.8615	1.1705	29
Y113G7A.5(n4331) V		5 cm	plate	4.952	1.286	50	4.3107	1.2817	33	2.0938	1.2025	36	4.4531	1.2848	38	2.7302	1.2124	30	1.4981	0.9445	18

Locomotion off Bacteria (pixels/second) | Well-fed Locomotion on Bacteria (pixels/second) | Food-Deprived Locomotion on Bacteria (pixels/second)

Table C.2

Normalized Mod Assay data. We calibrated our results using paired wild-type controls collected on every assay day. First wild-type control data from all days were pooled to create a master wild-type dataset. Our master wild-type data set was created using all wild-type locomotion data collected on 5 cm plates with animals food-deprived on plates. The master wild-type averages are listed at the top of the graph under 'Calibrated to' for each condition. A paired wild-type dataset was then created for each mutant, by pooling the wild-type control data for each day that specific mutant was assayed. To standardize data to the master control, we then multiplied the average speed of each mutant in each condition (off-food, well-fed, food-deprived) by the ratio between the master wild-type control and paired wild-type control (value Master / value Paired) for each condition. Standard error of the means were similarly calibrated by the same ratio of mean velocities.

Table C.2

Genotype	single trial	plate format	food deprivation	Calibrated to	SEM(L)	Calibrated to	SEM(B)	Calibrated to	SEM(E)
N2 - 5 cm/plate		5 cm	plate	5.103	0.033311287	4.0319	0.036854524	1.7367	0.029073686
age-1(hx546) II fer-15(b26) II		5 cm	plate	4.290569931	0.11200384	3.102433301	0.193309992	0.816967464	0.064778385
bas-1(ad446) III ; cat-4(e1141) V		5 cm	plate	3.676364078	0.081474575	4.661975391	0.105327155	2.847249294	0.115460083
bas-1(ad446) III ; cat-4(e1141) V		5 cm	liquid	3.765771858	0.113140493	5.373309166	0.127071671	2.866447989	0.167689108
C25G6.5(ok594) X		5 cm	plate	4.628417128	0.155898105	3.235484854	0.16372058	1.726508802	0.173333624
C30F12.6(ok1381) I		5 cm	plate	4.616511419	0.157113334	3.769978979	0.195006704	1.791552623	0.171489875
cat-2(e1112) II		5 cm	plate	5.018787141	0.120045634	6.122334942	0.153075242	2.96081454	0.23194884
cat-2(e1112) II		10 cm	liquid	4.640513992	0.106782496	5.362040943	0.123062346	4.731919368	0.170888215
cat-2(n4547) II		5 cm	plate	4.019167222	0.101403341	4.99523929	0.111208457	2.959651207	0.138381047
cat-2(n4547) II		5 cm	liquid	4.694554234	0.115019833	5.967661716	0.204218769	2.40628022	0.158031519
cat-2(n4547) II		10 cm	liquid	4.641909775	0.096230989	5.110431342	0.105125405	3.397200831	0.106093609
cat-2(n4547) II ; daf-2(e1370ts) III	*	10 cm	liquid	3.580708435	0.200973321	5.060342487	0.360549988	2.638030101	0.368015461
cat-2(n4547) II ; daf-28(sa191) V	*	10 cm	liquid	3.896217576	0.21307361	5.93985964	0.348929877	2.628391218	0.335707613
cat-2(n4547) II ; flp-21(ok889) V		10 cm	liquid	4.557182023	0.207823859	5.965542779	0.19566403	4.068797718	0.257604922
cat-2(n4547) II ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	plate	4.876052905	0.167590974	3.387024414	0.255443603	1.334124733	0.124104922
cat-2(n4547) II ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	liquid	4.764263675	0.173524054	3.098059361	0.214310041	1.340485524	0.09925161
cat-2(n4547) II ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		10 cm	liquid	4.66584805	0.133675906	4.13596521	0.218107361	1.872311756	0.17812843
cat-2(n4547) II ; mod-1(ok103) V		10 cm	liquid	3.76248137	0.144645805	3.951184045	0.188115849	1.602962286	0.131353873
cat-2(n4547) II ; mrp-1(pk89) X		5 cm	plate	4.136741377	0.164606904	4.809934667	0.179387185	1.559404207	0.20831346
cat-2(n4547) II ; mrp-1(pk89) X		10 cm	liquid	4.444096154	0.139053227	4.915800571	0.184381314	3.033388774	0.200551675
cat-2(n4547) II ; npr-1(ky13) X		5 cm	plate	4.884609443	0.150293977	5.774109375	0.213631575	3.860108689	0.275507537
cat-2(n4547) II ; npr-1(ky13) X		5 cm	liquid	4.724410538	0.163085046	5.625936903	0.219320687	3.64038852	0.270227869
cat-2(n4547) II ; npr-1(ky13) X		10 cm	liquid	4.788662903	0.110611185	5.147179671	0.105583686	5.288255392	0.104720054
cat-2(n4547) II ; tbh-1(n3722) X		5 cm	plate	3.920216695	0.136400391	2.997808196	0.180747118	1.100465966	0.101265368
cat-2(n4547) II tdc-1(n3420) II		5 cm	plate	4.259519655	0.107123144	4.12809655	0.186099786	1.138525379	0.096411557
cat-2(n4547) II tdc-1(n3420) II		5 cm	liquid	4.442240887	0.113490052	4.261493821	0.19375623	1.236532644	0.088459681
cat-2(n4547) II tph-1(n4622) II		5 cm	plate	4.433508047	0.080788044	4.733800091	0.123639914	2.162891046	0.14015356
cat-2(n4547) II tph-1(n4622) II		5 cm	liquid	4.317713611	0.118924528	4.229873811	0.214323739	2.05181759	0.167116706
cat-2(n4547) II tph-1(n4622) II		10 cm	liquid	4.393664488	0.132924872	4.368504417	0.148842647	2.232776393	0.149962163
cat-2(n4547) II tph-1(n4622) II tdc-1(n3420) II		5 cm	plate	5.589077714	0.106547297	4.613520896	0.103492124	1.688759517	0.151117905
CB4856 - Hawaiian wild-type strain		10 cm	liquid	6.549779526	0.158398346	6.039086155	0.138827284	4.089549838	0.14191969
che-1(p674) I		5 cm	plate	6.369428571	0.201291825	5.834567942	0.202334551	2.792899111	0.377492094
che-3(e1124) I		5 cm	plate	2.501505495	0.15444113	1.06041831	0.096382098	1.271297195	0.17025298
che-3(e1124) I	*	10 cm	liquid	2.825728963	0.203196321	2.006794112	0.241165658	1.563274548	0.199459649
che-3(e1124) I ; mrp-1(pk89) X	*	5 cm	plate	2.454849639	0.207413884	1.142183401	0.183912643	0.754434731	0.11995091
che-3(n1512) I		5 cm	plate	3.933296703	0.224233964	2.035869281	0.167341986	1.687627735	0.163495919
daf-1(m40ts) IV		5 cm	plate	4.50528938	0.130467366	4.146451005	0.246402875	2.073929075	0.175124518
daf-11(m47ts) V		5 cm	plate	4.726887426	0.116995647	1.544877677	0.141883905	1.404425141	0.091112324
daf-11(m47ts) V ; mrp-1(pk89) X		5 cm	plate	3.275238106	0.116411085	2.017347629	0.112503645	1.17942615	0.079128052
daf-11(m47ts) V ; npr-1(ky13) X		5 cm	plate	6.151419735	0.176332723	4.452534477	0.278863724	2.295675908	0.183941883
daf-12(m20) X		5 cm	plate	4.263002737	0.117719478	4.329027583	0.145173232	1.467371032	0.120430327
daf-2(e1370ts) III		5 cm	plate	3.000577283	0.088254584	1.732742014	0.083582105	0.776566395	0.064735312
daf-2(e1370ts) III	*	10 cm	liquid	4.604311377	0.263439851	2.656679806	0.212825814	0.99734094	0.151731829
daf-2(e1370ts) III ; mrp-1(pk89) X		5 cm	plate	3.231914517	0.123452679	1.537947365	0.146329461	0.688093885	0.074958639
daf-2(e1370ts) III ; npr-1(ky13) X		5 cm	plate	5.754122026	0.104419754	5.718546963	0.127997379	4.26281744	0.165150933
*daf-21(p673) V****		5 cm	plate	5.402787693	0.090544032	1.843894558	0.150255318	2.1502	0.127399952
daf-21(p673) V ; mrp-1(pk89) X		5 cm	plate	4.603537478	0.120288404	1.952642596	0.122302351	1.326503995	0.119654843
*daf-21(p673) V ; npr-1(ky13) X****		5 cm	plate	4.992854392	0.152455567	1.903673827	0.126784578	1.714973729	0.10657593
daf-28(sa191) V		10 cm	liquid	5.031930365	0.227735602	4.858591544	0.291797175	1.396693111	0.151583843
daf-3(e1376) X		5 cm	plate	4.585369835	0.097713475	3.513425138	0.166670884	1.52684495	0.1354491
daf-5(e1386) II		5 cm	plate	3.947588088	0.106106215	3.180153365	0.157200274	1.320069889	0.115746118

***strain may be incorrect

Locomotion off Bacteria (pixels/second) | Well-fed Locomotion on Bacteria (pixels/second) | Food-Deprived Locomotion on Bacteria (pixels/second)

Table C.2 - continued

Genotype	single trial	plate format	food deprivation	Calibrated to	SEM(L)	Calibrated to	SEM(B)	Calibrated to	SEM(E)
N2 - 5 cm/plate		5 cm	plate	5.103	0.033311287	4.0319	0.036854524	1.7367	0.029073686
daf-7(e1372ts) III		5 cm	plate	3.004800638	0.207617994	4.113059043	0.218896797	2.308100268	0.186940081
daf-7(e1372ts) III	*	10 cm	liquid	6.28295614	0.323034645	5.929138576	0.401371643	1.92361874	0.323534518
daf-8(e1393ts) I		5 cm	plate	4.542080195	0.136344513	3.775766156	0.290838157	2.100383723	0.142965262
dat-1(ok157) III		5 cm	plate	5.477433742	0.226093039	3.710666947	0.237637209	1.821743595	0.315838769
dop-1(vs100) X		5 cm	plate	5.024581255	0.182124783	3.654264601	0.223483449	1.303777057	0.183142363
dop-2(vs105) V		5 cm	plate	4.420672819	0.159786184	3.063341562	0.146253935	1.63865427	0.161958447
dop-2(vs105) V ; dop-3(vs106) X		5 cm	plate	4.988303275	0.204501024	2.950155747	0.214558429	2.05841176	0.248121693
dop-3(vs106) X		5 cm	plate	6.260359769	0.160678804	5.252099673	0.189833674	1.768482997	0.296189402
dop-4(ok1321) X		5 cm	plate	4.590948162	0.199480018	4.142735862	0.155978386	1.620162437	0.149388638
F14D12.6(ok371) X		5 cm	plate	4.730668637	0.181051025	2.898299621	0.153386753	1.227979425	0.139924964
F41E7.3(tm1497) X	*	5 cm	plate	5.227596156	0.345534321	3.276249017	0.423894718	1.208017938	0.173430588
flp-11(n4765) X		5 cm	plate	3.436561221	0.157986304	3.082396948	0.208503119	1.779461179	0.18338611
flp-18(n4766) X		10 cm	liquid	3.814726525	0.151255586	3.210686923	0.162602701	1.384003738	0.085363536
flp-18(n4766) X		5 cm	plate	4.939679348	0.096980876	3.922137873	0.194120989	1.702156382	0.124834572
flp-18(n4766) X flp-11(n4765) X	*	5 cm	plate	4.333312123	0.180076682	4.746100673	0.283718325	1.171217229	0.177948968
flp-21(ok889) V		5 cm	plate	5.207616755	0.113885928	4.87923371	0.14568091	3.03678165	0.163590339
flp-21(ok889) V		10 cm	liquid	4.868668388	0.206032373	4.596129845	0.188228935	1.930798436	0.120614272
flp-21(ok889) V ; flp-11(n4765) X		5 cm	plate	4.87383448	0.097124655	4.485572922	0.157599581	2.488301802	0.132022518
flp-21(ok889) V ; flp-18(n4766) X		5 cm	plate	4.803887382	0.107896287	5.079247257	0.168590578	1.935505502	0.16135625
goa-1(n1134) I		5 cm	plate	5.769939296	0.14778368	5.543241469	0.227291235	2.524983022	0.252799306
goa-1(n1134) I		10 cm	liquid	5.986251166	0.239779772	3.557348651	0.350536937	1.905880571	0.231650441
irk-1(n4895) X		5 cm	plate	5.499937511	0.143038913	4.217900094	0.202546452	1.764056338	0.153140734
irk-2(n4896) X		5 cm	plate	4.249769464	0.116716291	2.479610571	0.190969223	1.232428335	0.096295677
lin-15AB(n765ts) X ; nEx1242/3 (flp-11 overexp)		10 cm	liquid	4.984758561	0.136467153	1.860575333	0.151879488	0.999205521	0.07911708
lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	plate	4.750191828	0.117863766	3.310820795	0.2040536	1.253027643	0.106568526
lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	liquid	4.76141236	0.118686078	3.292386868	0.199054066	1.279633143	0.088341773
lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		10 cm	liquid	4.566433432	0.141833011	3.388231598	0.162759572	1.200439948	0.101206878
lin-15AB(n765ts) X ; nEx1277 (flp-18 overexp)		10 cm	liquid	5.032226686	0.158928227	3.815609236	0.126520276	1.891097184	0.120438922
mgDf50 I		5 cm	plate	3.170674486	0.125783057	2.757709695	0.128359274	1.208331015	0.104418901
mod-1(n3034) V		5 cm	plate	4.972771794	0.125727609	4.691990283	0.126260826	1.450808082	0.098956924
mod-1(n3034) V ; n4954		5 cm	plate	5.261418523	0.168926175	5.314687567	0.124240482	2.851269299	0.123256032
mod-1(n3034) V ; n4954		10 cm	liquid	5.161422083	0.117291026	4.232775468	0.120335543	2.39603964	0.100694588
mod-1(nr2043) V		5 cm	plate	4.927114536	0.153202403	4.336341325	0.122102701	1.53451197	0.099861762
mod-1(ok103) V		5 cm	plate	4.798555145	0.099686858	4.483153662	0.102059783	1.568865187	0.077702323
mod-1(ok103) V		5 cm	liquid	4.766402154	0.236646411	4.432030834	0.160009427	1.553104036	0.13059212
mod-1(ok103) V		10 cm	liquid	4.535534264	0.217041314	2.94504	0.179732659	1.18533972	0.112089598
mod-1(ok103) V ; lin-15AB(n765ts) X ; nEx1252		5 cm	liquid	4.170645249	0.132549387	2.633856083	0.203666686	1.188324227	0.073099438
mod-1(ok103) V ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	plate	4.187297231	0.117833262	2.688851065	0.229027312	1.152990187	0.073135737
mod-1(ok103) V ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		10 cm	liquid	4.169115422	0.140022833	2.612287538	0.184908824	1.258965367	0.098951047
mod-5(n3314) I		5 cm	plate	4.144573752	0.096081508	2.280302546	0.100456653	0.821320012	0.043070605
mod-5(n3314) I		5 cm	liquid	3.632757149	0.172471281	2.12230939	0.155918393	1.141338544	0.103193082
mod-5(n3314) I		10 cm	liquid	4.86327916	0.198827963	2.033661658	0.139889597	1.03085321	0.14367802
mod-5(n3314) I ; cat-2(e1112) II		5 cm	plate	4.535505495	0.148479832	4.14310249	0.29147595	1.114314177	0.10817112
mod-5(n3314) I ; cat-2(n4547) II tph-1(n4622) II tdc-1(n3420) II	*	5 cm	plate	2.624338513	0.323655149	4.319352533	0.631711681	0.992934314	0.179120729
mod-5(n3314) I ; daf-1(m40ts) IV		5 cm	plate	5.008981611	0.122124793	2.191555964	0.175974417	0.709133575	0.067401397
mod-5(n3314) I ; daf-11(m87) V		5 cm	plate	3.717325745	0.218940341	1.677050921	0.181841404	1.247608596	0.170346083
mod-5(n3314) I ; daf-2(e1370ts) III	*	10 cm	liquid	5.088986269	0.253491032	1.876381232	0.193431415	1.621411394	0.229280094
mod-5(n3314) I ; daf-7(e1372ts) III		5 cm	plate	4.409122839	0.217922368	1.361977535	0.190125205	0.887653876	0.127592051
mod-5(n3314) I ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	plate	2.639205596	0.121719275	1.016540523	0.07633142	0.846184109	0.077968166
mod-5(n3314) I ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	liquid	2.615702129	0.112627792	1.102442509	0.069820293	1.076567206	0.08133716

Locomotion off Bacteria (pixels/second) Well-fed Locomotion on Bacteria (pixels/second) Food-Deprived Locomotion on Bacteria (pixels/second)

Table C.2 - continued

Genotype	single trial	plate format	food deprivation	Calibrated to	SEM(L)	Calibrated to	SEM(B)	Calibrated to	SEM(E)
N2 - 5 cm/plate		5 cm	plate	5.103	0.033311287	4.0319	0.036854524	1.7367	0.029073686
mod-5(n3314) I ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		10 cm	liquid	3.009505187	0.133293281	1.167912571	0.097764435	1.154288475	0.108307109
mod-5(n3314) I ; npr-1(ky13) X		10 cm	liquid	5.818269713	0.171446319	4.855871405	0.177006219	2.440120122	0.121872158
mod-5(n3314) I ; tdc-1(n3420) II		5 cm	plate	2.44201581	0.171427635	1.120889569	0.118170121	0.595178785	0.111478964
mod-5(n3314) I ; tph-1(n4622) II		5 cm	plate	4.821908851	0.089920597	4.021981671	0.111027676	2.058433237	0.109833611
mod-5(n3314) I ; tph-1(n4622) II		5 cm	liquid	4.943252279	0.100627393	4.076618711	0.13701349	2.238201702	0.12949164
mod-5(n3314) I ; tph-1(n4622) II		10 cm	liquid	5.111550017	0.179249206	4.519907747	0.183250146	1.854248247	0.083462304
mod-5(n3314) I ; tph-1(n4622) II tdc-1(n3420) II	*	5 cm	plate	4.117445257	0.259274228	2.803958973	0.679671049	1.832431335	0.17908958
mod-5(n3314) I daf-8(e1393ts) I		5 cm	plate	4.886364472	0.150786824	2.601653725	0.194446764	0.659170601	0.071918154
mod-6(n3076) I		5 cm	plate	5.089967822	0.136710578	3.833159962	0.147876853	2.853442518	0.184794215
mod-6(n3076) I		5 cm	liquid	5.02058897	0.155969457	3.824567793	0.200951381	2.824726989	0.197799724
mod-6(n3076) I		10 cm	liquid	5.093725565	0.240868625	4.164870236	0.205283978	1.932989904	0.173705644
mrp-1(pk89) X		5 cm	plate	3.624684814	0.097520555	2.59876019	0.096644706	1.197574645	0.056965543
mrp-1(pk89) X		10 cm	liquid	3.903597395	0.174616173	2.511603938	0.18705587	0.8529768	0.090736103
mrp-1(pk89) X npr-1(ky13) X		5 cm	plate	6.18146201	0.177360424	5.470868747	0.152225969	4.305566555	0.192820473
n4954		5 cm	plate	5.646465078	0.128474986	5.104351954	0.12506082	2.492614597	0.12472067
npr-1(ky13) X		5 cm	plate	6.102052602	0.071794393	5.958398299	0.058909958	4.526436001	0.079351395
npr-1(ky13) X		5 cm	liquid	5.637608422	0.137859006	6.036916874	0.117576306	4.049874983	0.144790195
npr-1(ky13) X		10 cm	liquid	4.924643711	0.126458562	5.228609506	0.11042607	4.849135113	0.099318566
npr-1(ky13) X lin-15AB(n765ts) X ; flp-21 overexp		10 cm	liquid	5.980943598	0.144709148	5.148022945	0.213535712	5.169141505	0.154446832
npr-1(ky13) X lin-15AB(n765ts) X ; nEx1242/3 (flp-11 overexp)		10 cm	liquid	6.009317914	0.17367949	5.956722553	0.149324605	4.755520494	0.219481627
npr-1(ky13) X lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)	*	5 cm	plate	3.903307191	0.322500661	1.956315847	0.288161827	0.925660709	0.155598243
npr-1(ky13) X lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)	*	5 cm	liquid	3.893588348	0.323631544	1.802331935	0.222066733	1.15154775	0.149137469
npr-1(ky13) X lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		10 cm	liquid	4.579127005	0.142087674	3.323624715	0.192386497	1.162552483	0.07961425
npr-1(ky13) X lin-15AB(n765ts) X ; nEx1277 (flp-18 overexp)		10 cm	liquid	5.888052051	0.30199284	5.785668414	0.176195298	5.268460983	0.203137387
npr-1(ky13) X osm-1(p808) X	*	5 cm	plate	4.781852978	0.301604484	3.977310371	0.329597081	1.94978874	0.27315273
npr-1(ok1447) X		5 cm	plate	5.002575643	0.156946472	4.832196116	0.163127327	4.434029373	0.183054682
npr-1(ok1447) X	*	10 cm	liquid	6.532748457	0.317473164	6.071940238	0.212452782	4.863995457	0.262972333
npr-2(ok419) IV		5 cm	plate	2.586034064	0.137388534	0.807122412	0.068182336	0.77061492	0.08773405
npr-2(ok419) IV ; npr-1(ky13) X		5 cm	plate	3.144801999	0.164828974	2.032569849	0.206630758	1.553599643	0.166423996
npr-3(tm1583) IV	*	5 cm	plate	4.530390342	0.265220169	2.968749324	0.326677869	1.308498059	0.172006549
osm-1(p808) X	*	5 cm	plate	2.98221553	0.210861857	2.333043037	0.26180046	1.403681919	0.374135789
osm-1(p808) X		10 cm	liquid	3.974389043	0.247745395	1.848700258	0.174085233	1.58395346	0.127437763
osm-6(p811) V		10 cm	liquid	3.17693176	0.173537842	1.52187485	0.149126351	1.568115685	0.138563071
ser-1(ok345) X		5 cm	plate	5.512930008	0.185723001	4.267414515	0.178504776	1.814004633	0.244906569
ser-2(pk1357) X		5 cm	plate	5.585180678	0.169255013	3.644806362	0.156887294	1.636246467	0.174952204
ser-3(ad1774) I		5 cm	plate	4.731719827	0.212342212	3.413817062	0.168214515	1.519034627	0.250686677
ser-4(ok512) III		10 cm	liquid	5.266237368	0.173327826	4.346619238	0.184945373	1.754837056	0.132283338
ser-4(ok512) III (2x)		5 cm	plate	6.09781714	0.155174665	6.027045625	0.212764328	2.506652509	0.17955826
ser-7(n4542) X		5 cm	plate	5.301254362	0.180013038	3.501370858	0.17363072	0.929604503	0.121238084
T02E9.3(ok568) V		5 cm	plate	5.021178877	0.171167792	4.026007461	0.180709888	1.550737568	0.208788864
T21F2.1(n4330) X		5 cm	plate	4.196681644	0.170726967	3.65468394	0.138716531	1.674906061	0.180287622
T24D87(del) V		5 cm	plate	5.010564802	0.174753284	3.734560586	0.207912556	1.142814624	0.16077107
tax-2(p671) I		5 cm	plate	4.052273305	0.132037515	1.76122394	0.159849092	1.007551145	0.104712559
tax-2(p671) I		10 cm	liquid	4.785470312	0.191157101	2.344204893	0.211883762	1.470801358	0.134410953
tax-2(p671) I ; npr-1(ky13) X		5 cm	plate	4.649288867	0.150292439	2.811009914	0.193913398	1.45922986	0.190614635
tax-2(p671) I ; npr-1(ky13) X	*	10 cm	liquid	4.905481367	0.265478065	3.102272774	0.321299144	2.070295747	0.306158674
tax-4(p678) III		5 cm	plate	5.019888946	0.144409712	1.801790362	0.136762605	1.79584944	0.218513067
tax-4(p678) III		10 cm	liquid	5.445416168	0.1927102	2.71963279	0.191970739	1.717166744	0.153016565
tax-4(p678) III ; npr-1(ky13) X		5 cm	plate	4.764659391	0.152310466	2.643878791	0.186675805	1.503361493	0.159600214
tax-4(p678) III ; npr-1(ky13) X	*	10 cm	liquid	6.310475481	0.195448885	3.462885028	0.261396337	2.175271538	0.333581064

Locomotion off Bacteria (pixels/second) | Well-fed Locomotion on Bacteria (pixels/second) | Food-Deprived Locomotion on Bacteria (pixels/second)

Table C.2 - continued

Genotype	single trial	plate format	food deprivation	Calibrated to 5.103	SEM(L)	Calibrated to 4.0319	SEM(B)	Calibrated to 1.7367	SEM(E)
N2 - 5 cm/plate		5 cm	plate		0.033311287		0.036854524		0.029073686
tax-5(p672) V	*	10 cm	liquid	5.886490909	0.254753497	4.659049135	0.248768834	3.013356511	0.285498773
tbh-1(n3722) X		5 cm	plate	4.683480567	0.095991159	4.130704597	0.094154567	1.740719873	0.082755061
tdc-1(n3420) II		5 cm	plate	4.416144127	0.101878487	3.513061406	0.139483122	1.112350361	0.074895955
tdc-1(n3420) II		5 cm	liquid	4.385308712	0.107455588	3.520904108	0.144508952	1.154886977	0.05712841
tdc-1(n3420) II		10 cm	liquid	4.804259144	0.078206512	3.832185429	0.104403277	1.186087159	0.053604587
tdc-1(n3420) II ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	plate	3.653564551	0.108177934	1.618096222	0.130212192	0.873248807	0.071434312
tdc-1(n3420) II ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	liquid	3.616799128	0.107526333	1.580741476	0.105716608	1.133664456	0.070623059
tdc-1(n3420) II ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		10 cm	liquid	3.468787103	0.148802298	1.876935178	0.144761329	1.208466291	0.110088479
tdc-1(n3420) II ; npr-1(ky13) X		5 cm	plate	6.473380963	0.130574713	5.84404155	0.163251525	4.019928897	0.175052362
tdc-1(n3420) II ; npr-1(ky13) X		5 cm	liquid	6.504357946	0.129084964	5.849841985	0.163335298	3.972135167	0.1723108
tdc-1(n3420) II ; npr-1(ky13) X		10 cm	liquid	5.791248935	0.114405925	5.623707072	0.104090895	4.743979685	0.100899984
tph-1(mg280) II		5 cm	plate	5.054273141	0.101066482	4.250511884	0.109895921	2.320045209	0.112696364
tph-1(n4622) II		5 cm	plate	4.629913378	0.08087791	3.714777042	0.085694088	1.790657292	0.06406608
tph-1(n4622) II		5 cm	liquid	4.404658947	0.097711591	3.54294454	0.102763702	1.68245802	0.066371089
tph-1(n4622) II		10 cm	liquid	4.382631782	0.108955516	3.58542759	0.085912457	1.64444316	0.066943569
tph-1(n4622) II ; bas-1(ad446) III ; cat-4(e1141) V		5 cm	plate	4.564353094	0.114949793	4.027167723	0.142211128	2.298902494	0.151677721
tph-1(n4622) II ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	plate	4.393695766	0.125397726	2.868761042	0.185124145	1.481670994	0.088529239
tph-1(n4622) II ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		5 cm	liquid	4.371732525	0.125743184	2.843870274	0.176781798	1.497912175	0.076342299
tph-1(n4622) II ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp)		10 cm	liquid	5.104191614	0.134985468	3.455466501	0.154332115	1.608445725	0.119552036
tph-1(n4622) II ; mod-1(ok103) V	*	10 cm	liquid	4.339592396	0.209612169	4.196705981	0.229718045	1.755041117	0.149842327
tph-1(n4622) II ; mrp-1(pk89) X		10 cm	liquid	4.971644342	0.141638611	3.386954075	0.140460013	1.206753583	0.072483884
tph-1(n4622) II ; npr-1(ky13) X		5 cm	plate	5.279155513	0.147532099	5.148890065	0.171317299	4.220649121	0.143742832
tph-1(n4622) II ; npr-1(ky13) X		5 cm	liquid	5.278667102	0.1483636	5.147028809	0.171123513	4.196338104	0.142851794
tph-1(n4622) II ; npr-1(ky13) X		10 cm	liquid	5.086165194	0.168721182	5.10477079	0.13534198	4.695723607	0.123260098
tph-1(n4622) II tdc-1(n3420) II		5 cm	plate	4.850088471	0.098415898	4.410927956	0.133585612	1.939555933	0.085607334
tph-1(n4622) II tdc-1(n3420) II		5 cm	liquid	4.778223647	0.116266831	4.239638814	0.159420679	1.852741819	0.088188742
tyra-3(ok325) X		5 cm	plate	5.37641442	0.172409202	3.730277609	0.144677317	1.792849961	0.209340533
Y113G7A.5(n4331) V		5 cm	plate	4.588887177	0.21477744	2.553620846	0.20703666	1.242597321	0.184652554

Locomotion off Bacteria (pixels/second) Well-fed Locomotion on Bacteria (pixels/second) Food-Deprived Locomotion on Bacteria (pixels/second)

Table C.3

Normalized Mod Assay data from Table C.2 graphed. Data following each genotype indicates the size of plate assayed on and the method of food-deprivation. Shaded background represents wild-type mean speeds for comparison.

Table C.3

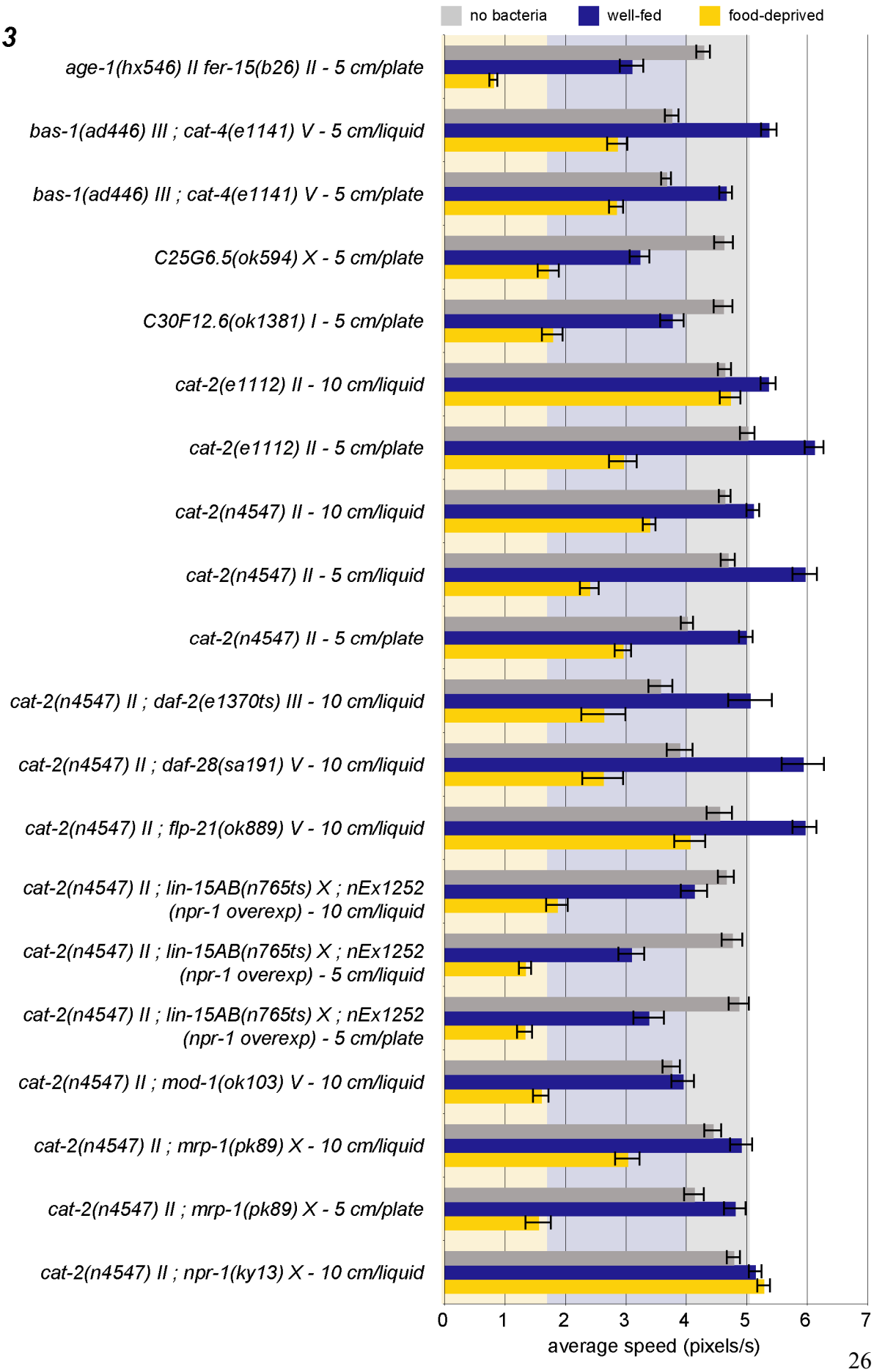

no bacteria
well-fed
food-deprived
age-1(hx546) II fer-15(b26) II - 5 cm/plate
bas-1(ad446) III ; cat-4(e1141) V - 5 cm/liquid
bas-1(ad446) III ; cat-4(e1141) V - 5 cm/plate
C25G6.5(ok594) X - 5 cm/plate
C30F12.6(ok1381) I - 5 cm/plate
cat-2(e1112) II - 10 cm/liquid
cat-2(e1112) II - 5 cm/plate
cat-2(n4547) II - 10 cm/liquid
cat-2(n4547) II - 5 cm/liquid
cat-2(n4547) II - 5 cm/plate
cat-2(n4547) II ; daf-2(e1370ts) III - 10 cm/liquid
cat-2(n4547) II ; daf-28(sa191) V - 10 cm/liquid
cat-2(n4547) II ; flp-21(ok889) V - 10 cm/liquid
cat-2(n4547) II ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp) - 10 cm/liquid
cat-2(n4547) II ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp) - 5 cm/liquid
cat-2(n4547) II ; lin-15AB(n765ts) X ; nEx1252 (npr-1 overexp) - 5 cm/plate
cat-2(n4547) II ; mod-1(ok103) V - 10 cm/liquid
cat-2(n4547) II ; mrp-1(pk89) X - 10 cm/liquid
cat-2(n4547) II ; mrp-1(pk89) X - 5 cm/plate
cat-2(n4547) II ; npr-1(ky13) X - 10 cm/liquid
0
1
2
3
4
5
6
7
average speed (pixels/s)

Table C.3 - continued

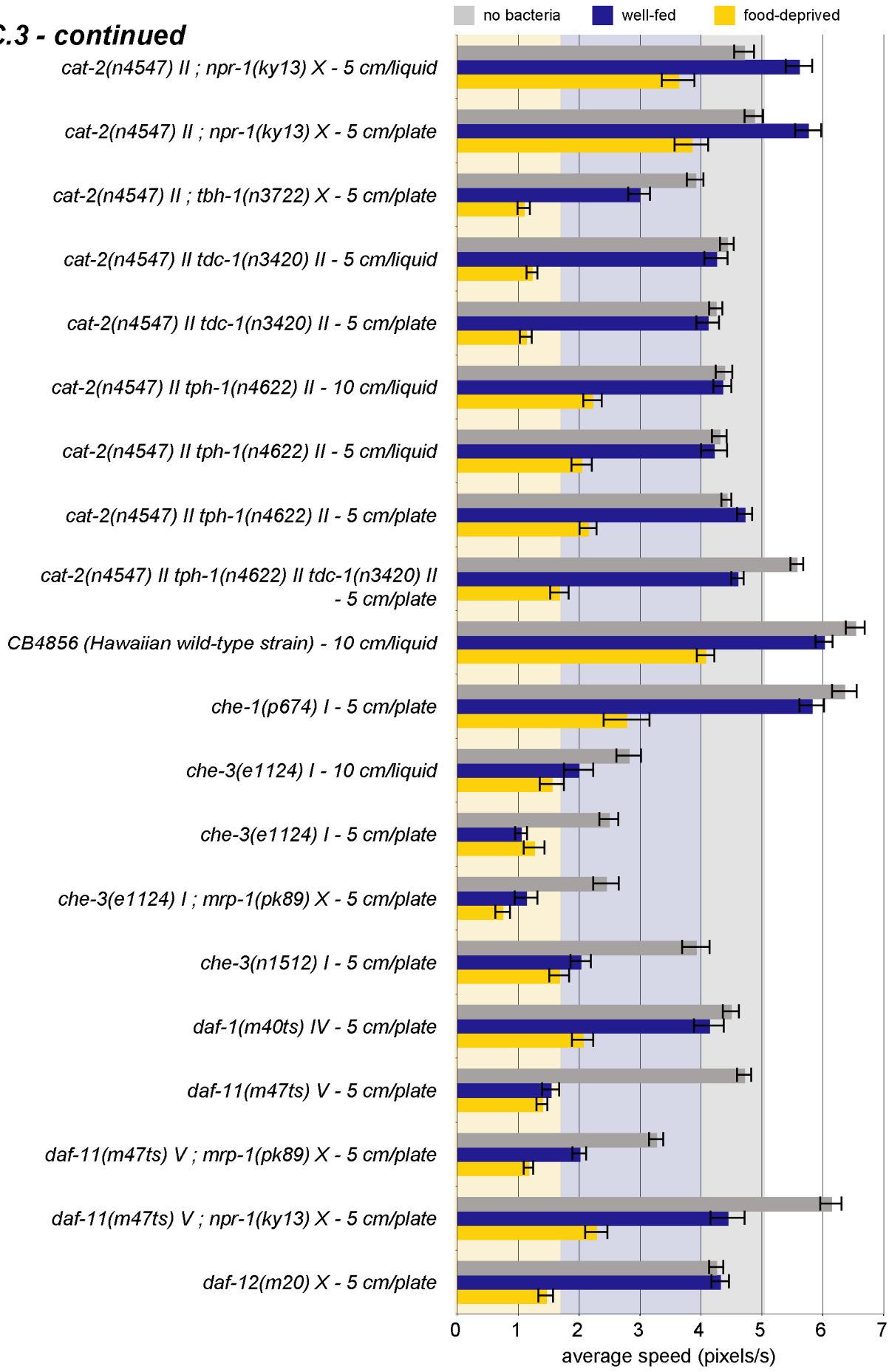
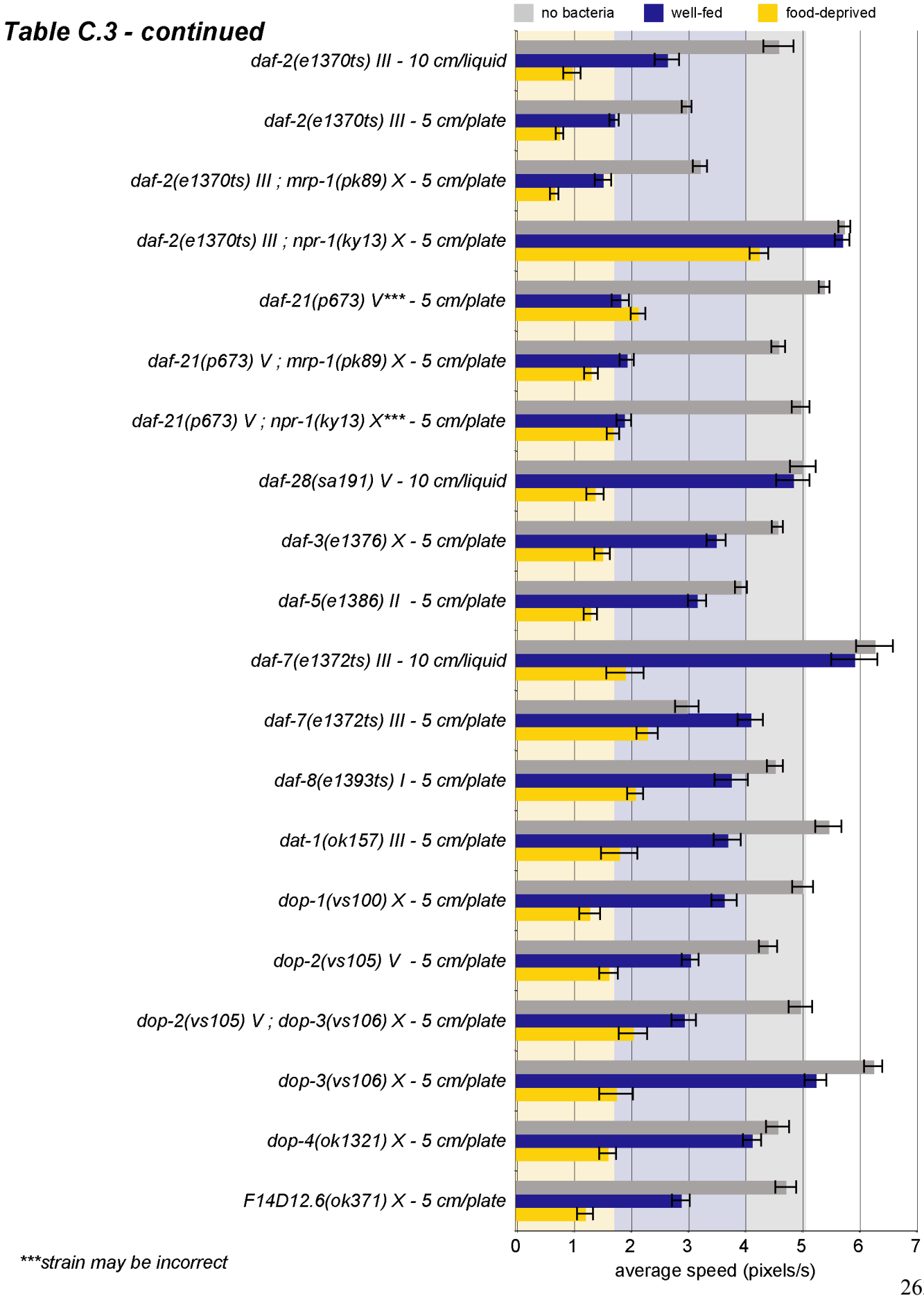


Table C.3 - continued



***strain may be incorrect

Table C.3 - continued

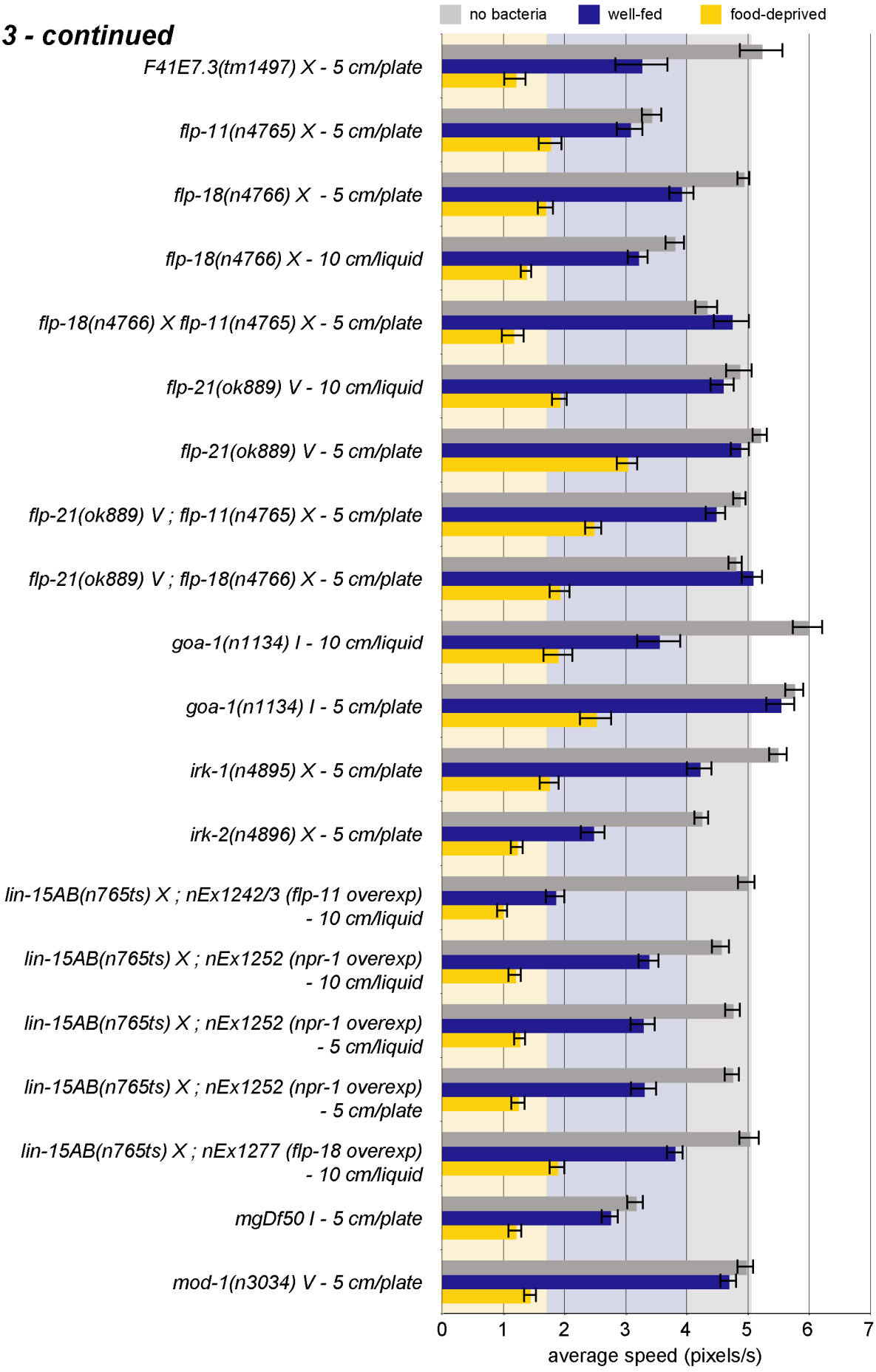


Table C.3 - continued

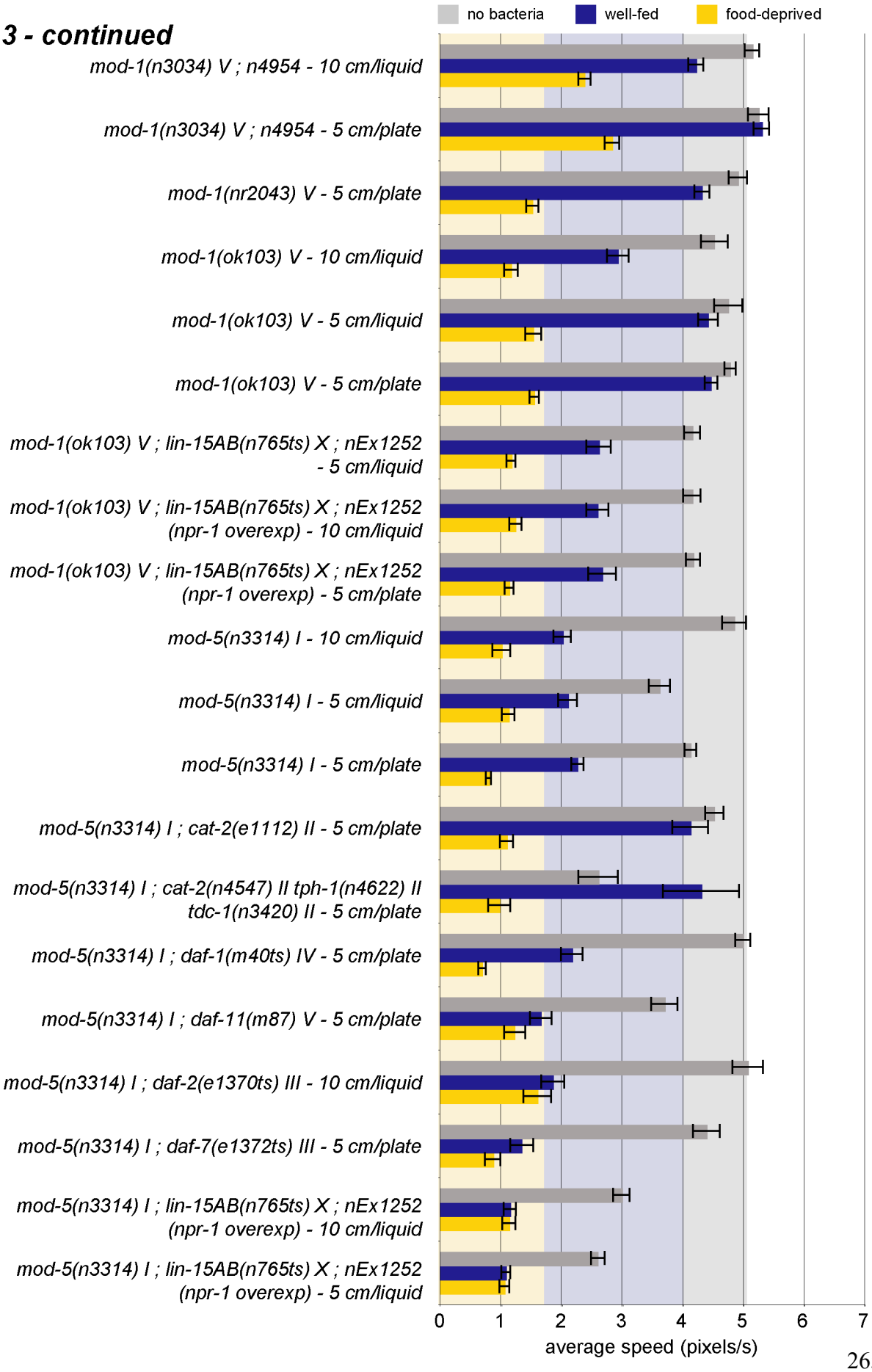


Table C.3 - continued

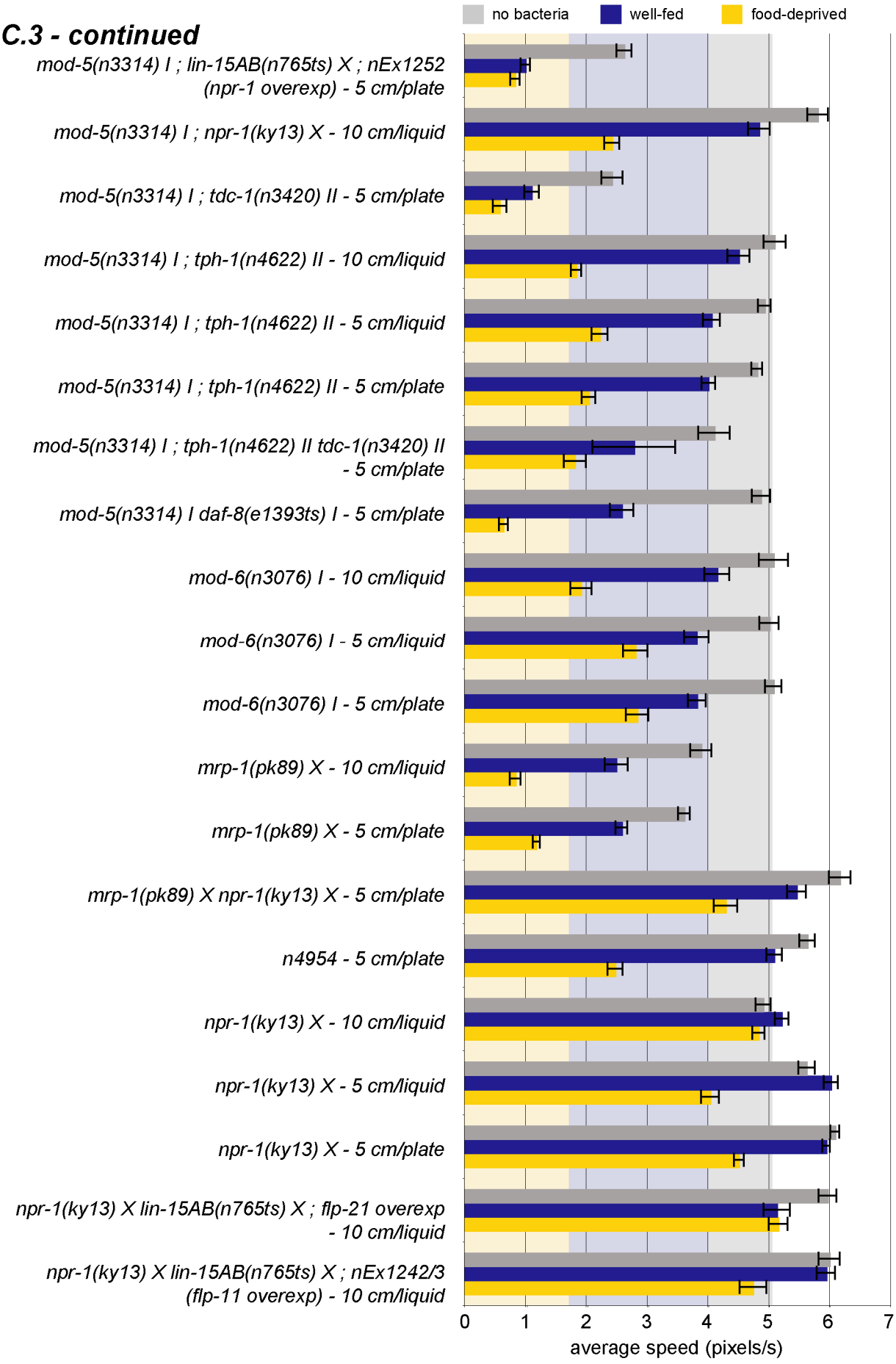


Table C.3 - continued

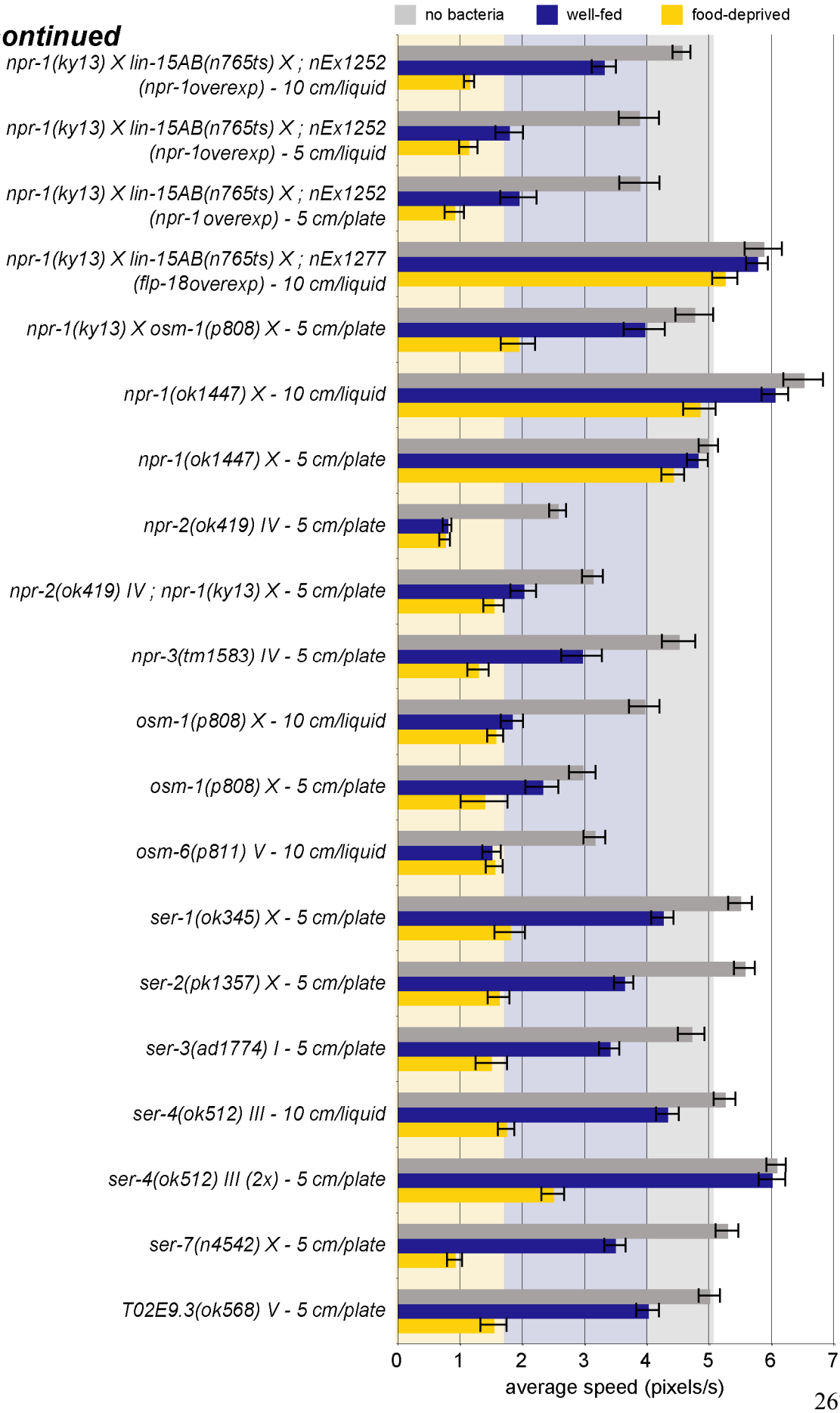


Table C.3 - continued

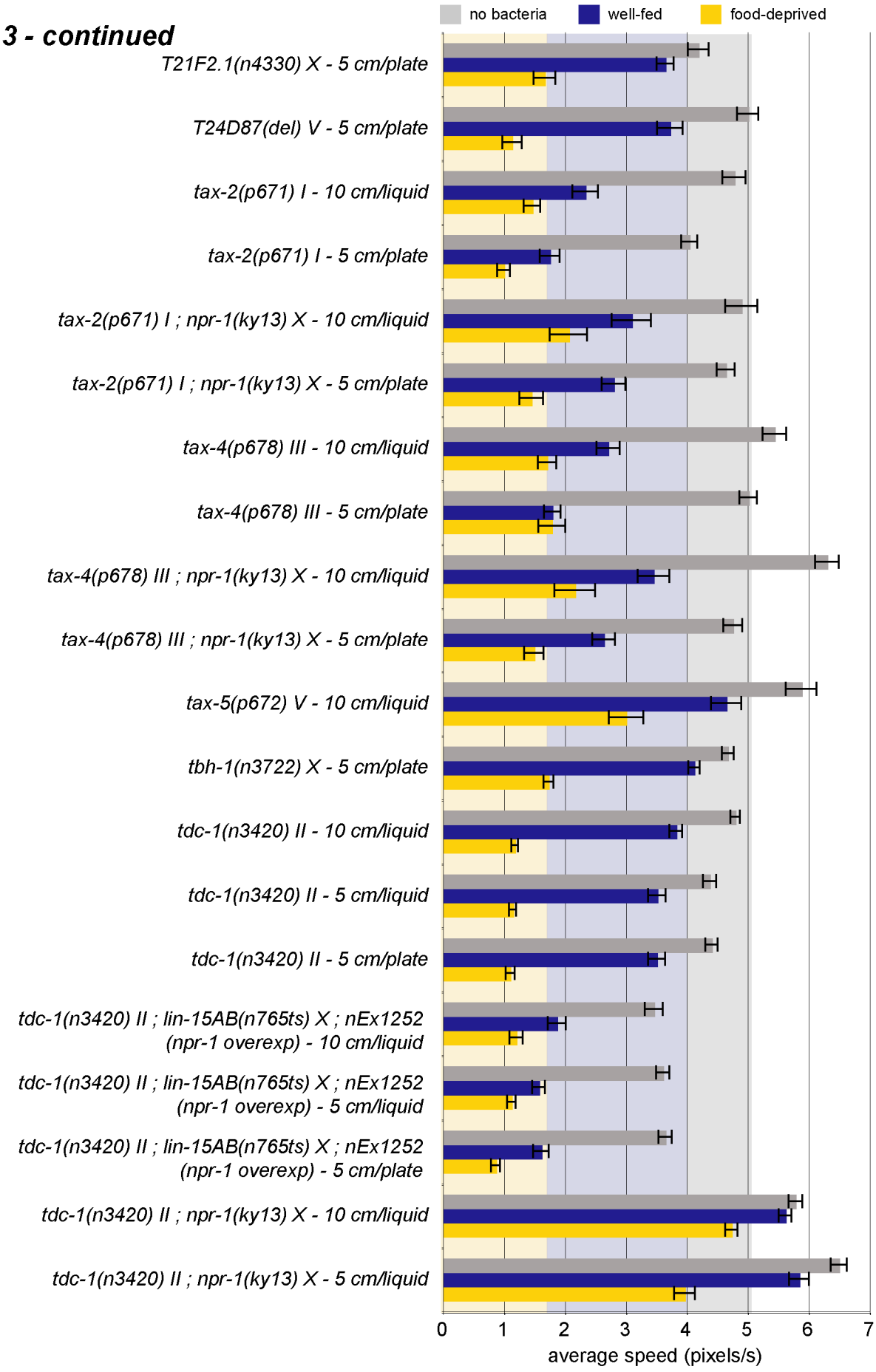


Table C.3 - continued