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THE GENETIC CHARACTERIZATION OF LOCOMOTIVE NEURAL CIRCUITS IN *CAENORHABDITIS ELEGANS*

by

AARON-JAY ALCALA

Under the Direction of Walter W. Walthall, PhD

ABSTRACT

Cellular networks are required for a variety of processes in complex organisms. *Caenorhabditis elegans* is a useful model to gain insight into the gene regulatory networks that assemble cellular networks. Mutations in a variety of genes can affect the sinusoidal locomotive pattern of *C. elegans*. We isolated the mutant *jd1500* from a standard genetic screen looking for mutants in *C. elegans* that exhibit asymmetric locomotive patterns. The two aims of this study were to: 1) identify the gene and characterize its role in the gene regulatory network and 2) characterize the cells affected by the mutation. We reasoned that *jd1500* likely disrupts the proper balance between dorsal and ventral body wall muscle contractions. By using three-point genetic mapping, we predicted the locus of *jd1500* between -9.42 and -11.73 centimorgans of the X chromosome. Our results implicate the embryonic, cholinergic DB motor neurons as likely cellular targets of the mutation.

INDEX WORDS: cellular networks, gene networks, locomotion, forward genetics, *Caenorhabditis elegans*

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by

AARON-JAY ALCALA

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science

in the College of Arts and Sciences

Georgia State University

2016

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2016

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College of Arts and Sciences

Georgia State University

December 2016

DEDICATION

To my parents, Luz and Willy Alcala, who have fully supported and motivated me throughout my studies. Regardless of being thousands of miles away the past years, you still encouraged me as if you were by my side. I hope my achievements make the sacrifices you've made worth it.

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I would like to thank Richard Campbell for his support and mentorship throughout my lab experience. You encouraged me to think critically to tackle challenging problems and you promoted a lively work environment. Special thanks to my other lab members, past and present, for their support: Christian, Michael, Kendra, Ling, Sam, Jasmine, Crystal, Mohammad, Anela, and Shelley. You created a superb atmosphere that made working in the lab a fun experience.

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1. INTRODUCTION

1.1 Cellular networks maintain biological processes and behaviors

In multicellular organisms, cellular networks are formed to maintain biological processes and give rise to complex behaviors. Cellular networks are composed of cells, but require DNA, proteins, and additional molecules that work in concert throughout the lifetime of an organism (Qi & Ge, 2006). The mammalian heart is an organ composed of cellular networks whose assembly is orchestrated by signaling pathways and networks of transcription factors during cardiogenesis. Defects that occur during the development of the heart often lead to congenital heart disorders (Bruneau, 2013). During development, cardiomyocytes form an electrical conduction system in the heart which stimulates the atrial and ventricular contractions that occur throughout life. The chambers of the heart must maintain precise rhythmic contractions in order to transport blood in a coordinated manner (Silverman & Hollman, 2007). The neural network of the autonomic nervous system interacts with the myocardial network to add an additional level of control of heart rate when the body needs enhanced or decreased blood flow (Levy, 1972).

Swimming, crawling, flying, and walking are behaviors which also require coordinated rhythmic control of muscle contractions. In many animal species, rhythmic motor behaviors are controlled by interneuronal networks called central pattern generators (CPGs) (Brown, 1911). CPGs create rhythmic muscle contractions coordinated by proprioceptive receptors, which detect muscle length and muscle tension (Wen et al., 2012). During locomotion, muscular proprioceptors regulate timing and intensity of muscle contractions in response to the speed of locomotion (Rossignol et al., 2006). Disrupting sensory feedback from proprioceptors in muscle spindles impairs the locomotive pattern in mice (Akay et al., 2014). How proprioceptive networks interact with the central nervous system to give rise to proper locomotion is not fully understood.

Manipulating essential organs like the heart in vertebrates to understand more about the cellular networks that maintain coordinated motor programs is difficult due to the intrinsic complexity of the neuromuscular networks. For this reason, using a simpler organism like *Caenorhabditis elegans* (*C. elegans*) can give insight into how cells are organized into networks. Although simple, the nematode shares similar types of tissues and behaviors with more complex animals. Of the 959 somatic cells that *C. elegans* hermaphrodites develop, 302 are neurons. The ventral nerve cord (VNC) of the nematode contains 75 motor neurons (mns) that innervate dorsal and ventral body wall muscles that control locomotion (White et al., 1986). In contrast, the human brain alone is estimated to have an average of 86 billion neurons (Azevedo et al., 2009).

1.2 *C. elegans* as a genetic model

Complex cellular networks can be better understood by studying the gene regulatory networks (GRNs) that assemble them. In 1965, Sydney Brenner established *C. elegans* as a model system to study the genetics of development and behavior (Brenner, 1973, 1988). *C. elegans* are transparent and grow to about 1.5 mm as adults. After hatching from eggs, nematodes go through four larval stages (L1, L2, L3, and L4) and mature into egg-laying adults in only about 3 days (Figure 1). Thousands of worms can be grown on solid media in a petri dish with *E. coli* as a source of food. The worms exist either as self-fertilizing hermaphrodites or cross-fertilizing males. This allows convenient manipulation for genetic crosses (Riddle et al., 1997). The *C. elegans* genome is fully sequenced and contains genes homologous with other species (The *C. elegans* Sequencing Consortium, 1998).

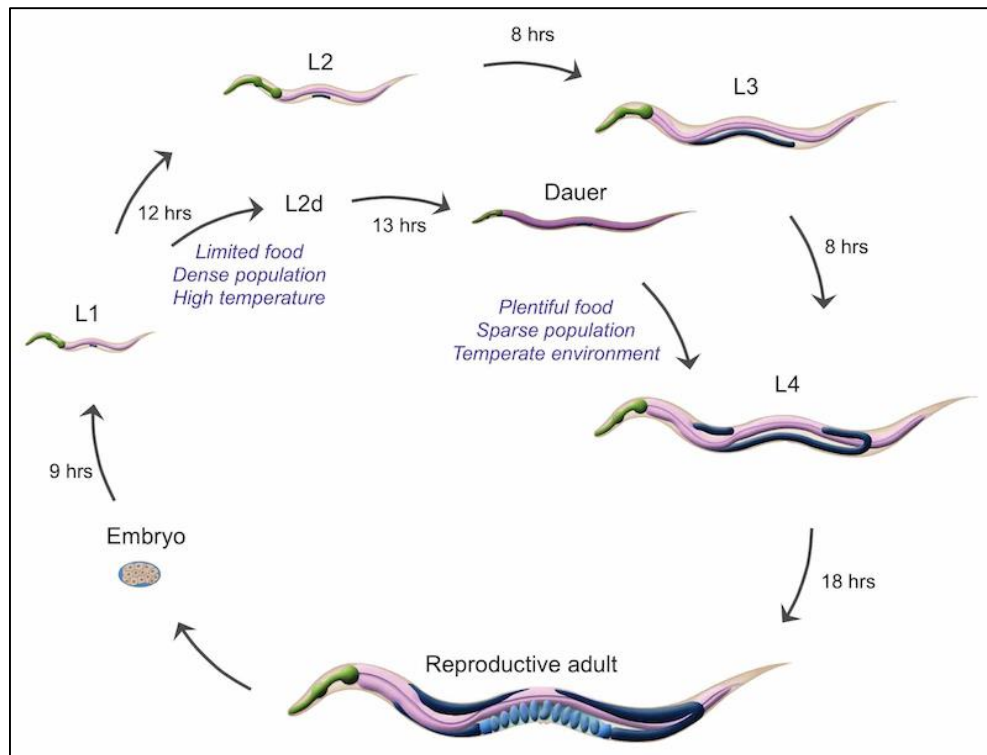


Figure 1. *C. elegans* life cycle.

C. elegans worms go through 4 larval stages (L1, L2, L3, and L4) after hatching from eggs. After the L4 larvae molt, the hermaphrodites mature into reproductive adults that can survive for approximately 3 weeks under normal laboratory conditions. If L1s grow in harsh environmental conditions, they enter an alternative larval stage called dauer that can survive up to 4 months. When returned to favorable environmental conditions, dauer larvae molt into L4 larvae and resume normal development (Wolkow & Hall, 2015).

The forward genetic screen was pioneered in *C. elegans* by Sydney Brenner (1974). In his study, he exposed wild-type hermaphrodites to ethyl methanesulfonate (EMS), a mutagen used to create mutations in germline cells of the animals. He then screened the F2 progeny for worms displaying mutant phenotypes. If the mutants produced viable progeny, the strain would be studied to identify the gene causing the phenotype and characterize the cells affected by the mutation. In his forward genetic screen, he isolated about 300 mutants affecting behavior or morphology and identified about one hundred mutated genes. Today, emerging technologies of whole-genome analysis have been used in conjunction with forward genetic techniques in order to better study gene regulatory networks in *C. elegans* (Jorgensen & Mango, 2002). The use of forward genetics in *C. elegans* has led to the identification of genes involved in the development and maintenance of cellular networks, and

continues to be important in solving biological problems in more complex organisms. Many of the genes and gene networks have been conserved.

1.3 The neuromuscular network behind *C. elegans* locomotion

Since *C. elegans* has a relatively simple anatomy and is easy to manipulate, it is useful to study the genetic basis of neurodevelopment and the generation of patterned behavioral outputs such as locomotion. As in more complex organisms, locomotion in *C. elegans* is mediated by interactions between neural and muscular networks. Alternating ventral and dorsal body wall muscles contractions thrust the worms forward or backward. The body wall muscles are innervated by five classes of motor neurons of the ventral nerve cord (VNC): A, B, D, VC, and AS (Table 1). The A-, B-, VC-, and AS-type neurons are excitatory and release acetylcholine (ACh), whereas the D-type neurons are inhibitory and release γ -aminobutyric acid (GABA) (Richmond et al., 1999; Lewis et al., 1980; McIntire et al., 1993). The A, B, and D classes are further subdivided based on whether they innervate ventral or dorsal muscle (VA, DA, VB, DB, VD, and DD) and whether they are born during embryonic or postembryonic stages.

Table 1. Motor neuron classes in *C. elegans*.

ACh = Acetylcholine, GABA = γ -Aminobutyric acid.

Motor Neuron Class	Locomotion involved in	Neurotransmitter	Origin
DB	Forward	ACh	Embryonic
VB	Forward	ACh	Postembryonic
DD	Forward/ Backward	GABA	Embryonic
VD	Forward/ Backward	GABA	Postembryonic
DA	Backward	ACh	Embryonic
VA	Backward	ACh	Postembryonic
VC	Egg-laying behavior	ACh	Postembryonic
AS	Unknown	ACh	Postembryonic

Interactions between interneurons and motor neurons regulate the alternating ventral and dorsal muscle contractions that thrust the animal forward or backward (Figure 2A). The A-type motor neurons are responsible for backward locomotion and receive input from the AVA, AVD, and AVE interneurons, whereas the B-type motor neurons are responsible for forward locomotion and receive input from the AVB and PVC interneurons (Chalfie et al., 1985; White et al., 1986). The A- and AS-type neurons have anteriorly directed processes while the B-type neurons have posteriorly directed processes. The D-type neurons participate in backward locomotion and, to a lesser extent, forward locomotion by acting as cross-inhibitors that prevent the dorsal and ventral muscles at the same cross-sectional level from contracting simultaneously (McIntire et al., 1993). The DD neurons are postsynaptic to the VB and VA neurons, whereas the VDs are postsynaptic to the DB, DA, and AS neurons (White et al., 1976, 1986).

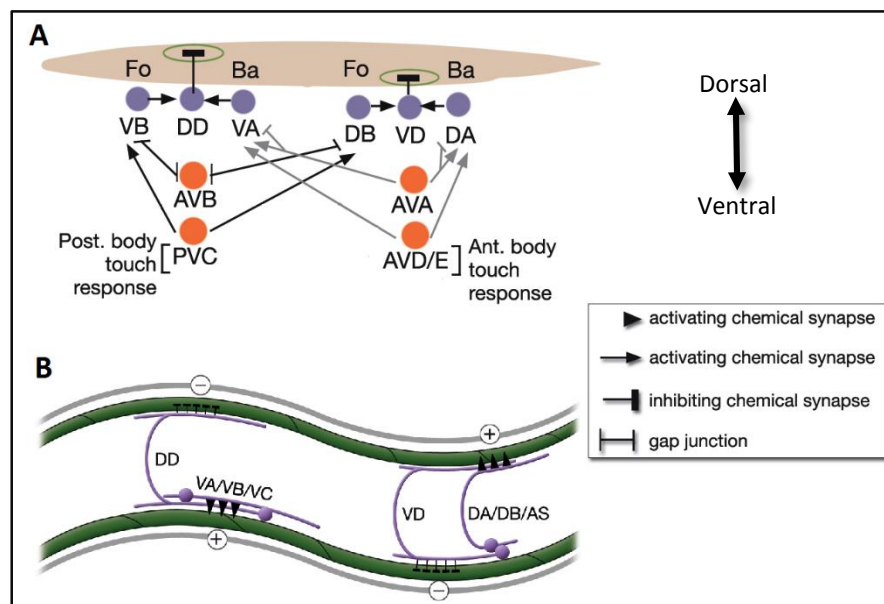


Figure 2. Locomotive neural circuit in *C. elegans*.

A) Five mechanosensory neurons (not shown) detect touch stimuli and synapse onto command interneurons (orange). These interneurons synapse onto motor neurons (purple) which control forward (Fo) and backward (Ba) locomotion. Green circles represent muscle.

B) The inhibitory DD and VD neurons receive inputs from the A- and B-type neurons opposite to where they innervate muscles. When muscles on one side of the animal contracts (+), the muscles on the other side relax (-) (modified from Altun & Hall, 2011).

The motor neurons innervate longitudinal bundles of body wall muscles organized into four quadrants along the anteroposterior axis of the animal (White et al., 1986). Eighty-one of the muscle cells that make up the body wall muscles are born embryonically, while the remaining fourteen develop post-embryonically (Sulston and Horvitz, 1977; Sulston et al., 1983). Adjacent muscle cells are electrically coupled by gap junctions (Liu et al. 2006). On solid agar, the worms move in a sinusoidal pattern generated by alternating ventral and dorsal body wall muscle contractions that propagate along the anteroposterior axis (Gray & Lissmann, 1964). The D-type neurons ensure that when dorsal muscle contracts, the ventral side relaxes and vice versa (Figure 2B). The overall magnitude of the dorsal and ventral contractile waves must be equal for normal locomotion.

1.4 The gene regulatory networks that give rise to neural networks

Studying the genetic programs that assemble diverse cell types in *C. elegans* can give insight into understanding the organization of gene regulatory networks responsible for the assembly of nervous systems in more complex animals. In *C. elegans*, *unc-4*, *vab-7*, and *ceh-12*, have been identified as transcriptional repressors of motor neuron-specific genes that distinguish DA from DB neurons and VA from VB neurons (Winnier et al., 1999; Esmaeili et al., 2002; Von Stetina et al., 2007). The terminal selector gene *unc-3* is required for the expression of a battery of genes involved in motor neuron differentiation (Kratissios et al., 2012). The transcription factor UNC-3 likely targets *unc-4* and *vab-7*, leading to their expression in DA and DB neurons (Kratissios et al., 2012). Mutations in a variety of genes required for motor neuron differentiation can affect proper locomotion in *C. elegans*.

1.5 Aims of study

Interestingly, forward locomotion in *C. elegans* is less often affected by genetic mutations compared to backward locomotion. Mutations in several genes are predicted to disrupt forward locomotion, but instead only affect backward locomotion. The gene *unc-25* encodes glutamic acid decarboxylase, an enzyme required for GABA synthesis (McIntire et al., 1993). *unc-30* encodes a

homeodomain transcription factor required for the terminal differentiation of the D-type motor neurons (Jin et al., 1994). In both *unc-25* and *unc-30* mutants, the GABAergic D-type neurons do not function properly. Although *unc-25* and *unc-30* mutants move backward poorly, they can move forward well. *unc-55* encodes a nuclear hormone receptor that distinguishes the DD and VD neurons during development (Zhou & Walthall, 1998). In *unc-55* mutants, the VD motor neurons adopt the synaptic pattern of the DD motor neurons, leading to increased inhibition of the dorsal muscle (Walthall and Plunkett, 1995, Shan et al, 2005). This causes the animals to move backward poorly with a ventral bias, but does not affect forward locomotion.

In this thesis, we aim to investigate the robustness of forward locomotion by characterizing a mutant generated in a forward genetic screen. Following ethyl methanesulfonate (EMS) mutagenesis, we isolated an X-linked recessive mutant (*jd1500*) with an unusual and interesting locomotion defect when moving forward. When prodded on the tail, the body of the mutant animals assume a coil-like shape as they move forward. When prodded on the head, the animals move backwards in the typical sinusoidal pattern. The *jd1500* mutant coils at the early L1 stage and into adulthood, and usually shows a ventral bias when attempting to move forward.

The two major aims of this study are to: 1) identify the genetic locus of the mutation and characterize its role in the gene regulatory network and 2) characterize the cells affected by the mutation. We hypothesize that *jd1500* is a mutation in a gene involved in maintaining the proper balance between dorsal and ventral body wall muscle contractions. The ventral bias exhibited by *jd1500* during forward locomotion at the early L1 stage implicates the embryonic, cholinergic DB motor neurons as the cellular target. We predict that this mutation is disrupting the proper balance of acetylcholine release between dorsal and ventral muscles. The mutant phenotype could be due to a physiological, anatomical, or developmental disruption.

2. MATERIALS AND METHODS

2.1 Strain maintenance and matings

The following strains were used: *dgk-1* (sy428), *dop-1* (vs101), *dpy-6* (e14), *fax-1* (gm83), *fkh-2* (ok683), *lon-2* (e678), *sax-3* (ky123), *snt-1* (md290), *unc-2* (e55), *unc-20* (e112), *unc-25* (e156), *unc-4* (e120), *unc-42* (e270), *unc-7* (e5), *unc-7; lon-2* (e139, e678), *unc-78* (e1217), and *+/szT1 [lon-2]; syDf1/szT1* (HR890). These were obtained from the Caenorhabditis Genetics Center. *punc-129::gfp* (ER80) was previously generated in our laboratory. The N2 Bristol strain was used as the wild-type strain. All worm strains were grown and maintained on NGM plates with a lawn of OP50 *E. coli* at 22°C as described by Brenner (1974).

Since *jd1500* is X-linked and mutant males are able to mate, they were used in matings for complementation tests and to generate double mutants. In order to generate *jd1500* males, five N2 males were placed on a plate with two *jd1500* hermaphrodites. The F1 was screened for males expressing the coiler phenotype. For crosses involving *jd1500* males, ten *jd1500* males were placed on a plate with two hermaphrodites to improve mating outcomes.

In order to generate double mutants, *jd1500* males were mated with mutants of interest and the F1 was screened for hermaphrodites with wild-type locomotion. These wild-type hermaphrodites were isolated to generate self-progeny and the F2 generation was screened for double mutants. To perform complementation tests, candidate mutant L4 hermaphrodites were placed on a plate with *jd1500* males. The F1 cross progeny were screened for hermaphrodites showing either wild-type or uncoordinated locomotion patterns.

2.2 Mutagenesis with ethyl methanesulfonate (EMS)

The mutagenesis protocol was performed as described by Brenner (1974). A plate containing N2 worms was washed into a 15 ml tube and washed two more times to remove bacteria. Animals were resuspended in 2 ml of M9 and 2ml of stock 100 mM EMS was added to the tube. The tube was incubated at 22°C for four hours. The animals were then washed five times to remove traces of EMS. A glass pipette was used to transfer worms to large plates containing NGM. The F2 progeny was screened for worms expressing uncoordinated locomotion patterns. The *jd1500* mutant isolated was outcrossed three times before further analyses.

2.3 Locomotion and thrashing assays

Since presence of eggs in adults may affect locomotive behavior, L4s were used in movement assays. Worms were transferred to an unseeded plate and allowed to crawl for five minutes to remove excess bacteria from their bodies. Each mutant worm was then prodded with a platinum wire on the tail. The worm was observed to determine if it initially entered a ventral or dorsal coiling position. Repeated prodding was avoided to prevent habituation to touch stimulus. Thrashing rate was recorded as described by Chou et al., (2015). Individual worms were placed into 50 μ l of M9 medium and allowed to acclimate to the liquid environment for one minute. The thrashing rate was counted for 30 seconds for each worm. One thrash was counted when the head of the worm performed a lateral movement.

2.4 Confocal microscopy

Animals were synced to be imaged at the early- to mid-L1 stage by transferring eggs to new plates up to 8 hours before collecting images. Animals were mounted on a 10 mM sodium azide 2% agar pad. Images of L1 animals were taken with 40x oil immersion objective. A Zeiss LSM 500 confocal microscope was used to image animals expressing GFP and the LSM Image Browser v4.2 was used to analyze images.

2.5 Aldicarb-sensitivity assays

Aldicarb-sensitivity assays were performed blind as described by Mahoney et al. (2006). Thirty L4 larval stage animals of each genotype were transferred to plates seeded with OP50. Twenty-four hours later, 25 animals from each plate were transferred to 1.0 mM aldicarb plates. Each strain was scored every 30 minutes for the ratio of animals moving to animals paralyzed. Animals were considered paralyzed if they did not move after being prodded three times with a platinum wire on both the head and tail, and if pharyngeal pumping was ceased. The assay was repeated three times on sequential days using each strain.

2.6 Genetic mapping (three-point mapping)

Mapping strains used were *lon-2* (*e678*) located at X: -6.74 and *dpy-6* (*e14*) located at X: 0.00. Hermaphrodites of each mapping strain were mated with *jd1500* males. Wild-type hermaphrodites in the F1 were isolated and allowed to generate self-progeny. The F2 was screened for double mutants resulting from recombination events.

Once double mutants were obtained, these were mated with N2 males. Wild-type hermaphrodites in the F1 were isolated and allowed to generate self-progeny. Each worm in the F2 generation was screened for its phenotype in order to calculate the recombination frequency.

2.7 Deficiency mapping

The *jd1500* mutant was mated into the deficiency strain: *+/szT1 [lon-2]; syDf1/szT1* (HR890). The region of the deletion spans from -11.73 to -4.24 centimorgans on the X chromosome. Since *jd1500* males are capable of mating, *jd1500* males were mated with hermaphrodites of the deficiency strain. Hermaphrodites in the F1 generation were screened for their locomotive phenotype and the severity of any locomotive defect.

3. RESULTS

3.1 Locomotive behavior analysis

In a forward genetic screen aiming to isolate mutants displaying biases in locomotion, we isolated *jd1500*, which demonstrated a locomotion defect when moving forward. When prodded on the tail, the head of the mutant turns sharply and the body enters a coil-like shape as they attempt to move forward (Figure 3). When prodded on the head, the animal moves backward in the typical sinusoidal pattern. The *jd1500* mutant demonstrates the coiler phenotype at all larval stages and into adulthood.

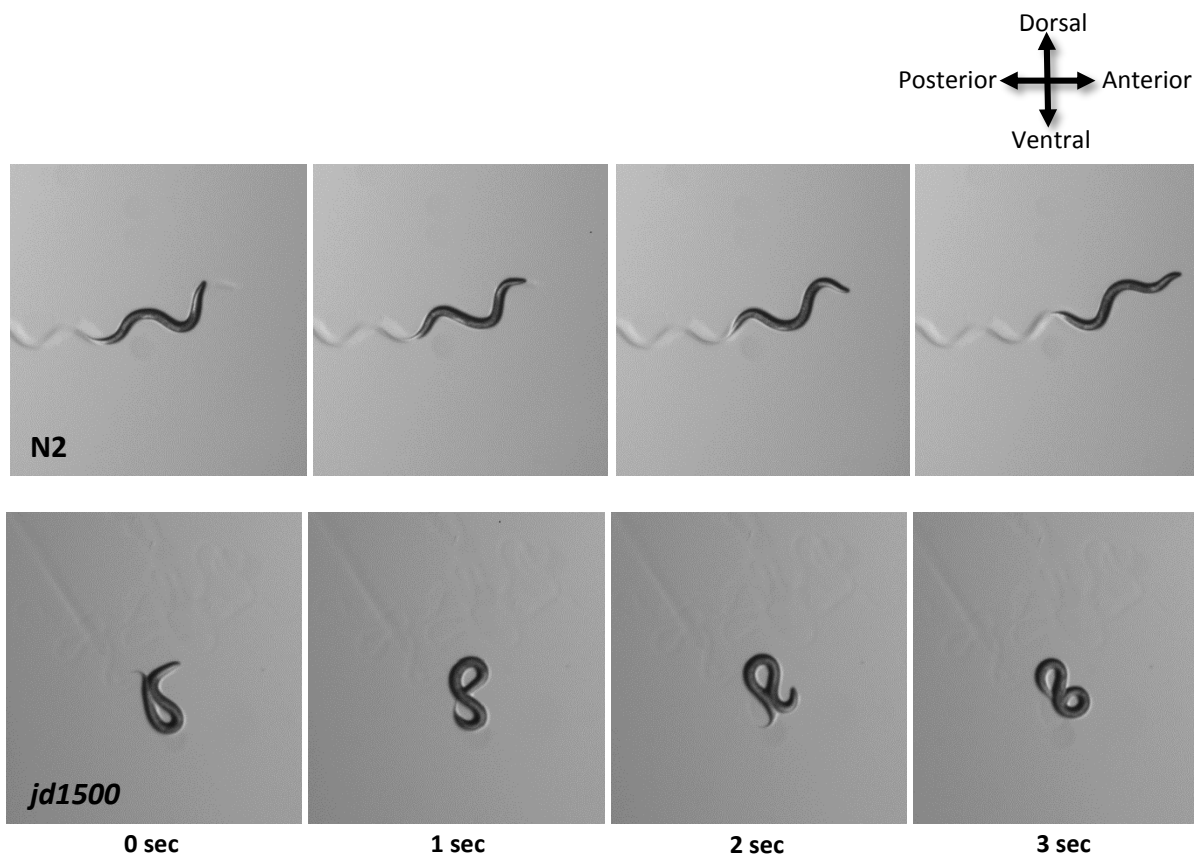


Figure 3. Forward locomotion in *jd1500* mutants in response to touch.

The *jd1500* mutant initially enters a ventral coil at 1 sec. then assumes a dorsal coil by the 3 sec. time point. Animals were imaged at the L4 stage.

To characterize the locomotive behavior of *jd1500* mutants, L4 animals were observed for a ventral or dorsal bias when coiling forward immediately after being prodded with a platinum wire. The animals tended to enter a ventral coil when moving forward initially, but also enter a dorsal coil in 28 percent of the animals observed (Figure 4A). We also analyzed the thrashing behavior of mutants in M9 solution. The *jd1500* mutants thrashed an average of 40 fewer lateral head movements than wild-type animals (Figure 4B). When attempting to thrash, the mutant tends to remain in a coil-like position for a couple of seconds before performing another lateral head movement.

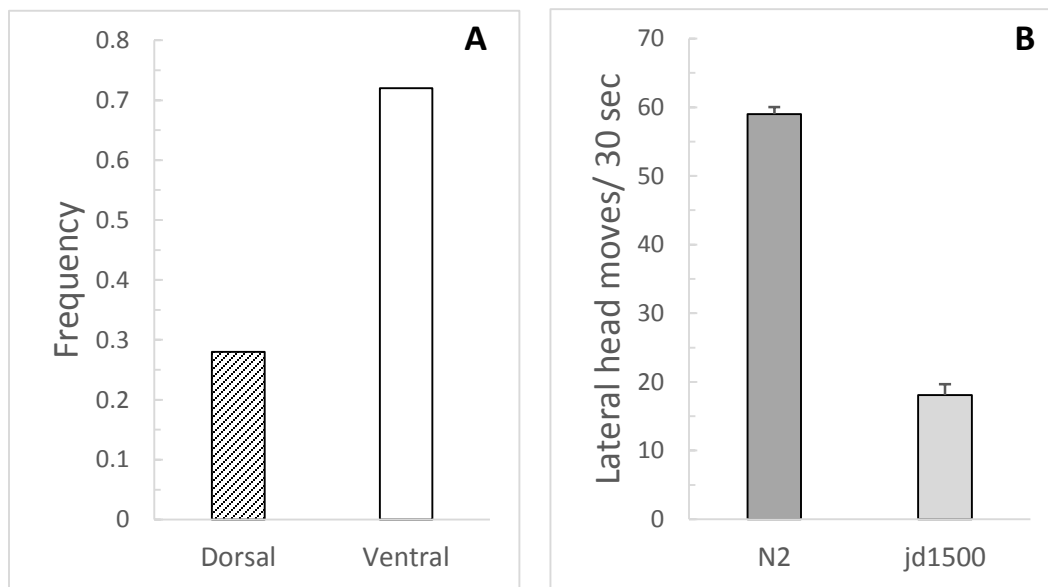


Figure 4. Movement assays.

A) Frequency of dorsal versus ventral coiling in *jd1500* mutants (n=50).

B) Frequency of thrashing in in wild-type and *jd1500* animals (n=20). $p < 0.001$; significance was calculated using Student's t-test. Error bars represent SEM.

3.2 Comparison of DA and DB neurons in wild-type and *jd1500* mutants using *punc-129::gfp* reporter gene expression

To determine if the DA and DB neurons were present in *jd1500* mutants, we introduced *jd1500* into a *punc-129::gfp* (ER80) background. *unc-129* encodes a member of the TGF- β signaling molecules (Colavita & Culotti, 1998). In *punc-129::gfp* animals, green fluorescent protein (GFP) expression is detected in the DA and DB motor neurons in all larval stages and persists into adulthood (Colavita et al., 1998). Ten *jd1500* animals were observed in the *punc-129::gfp* background (data not shown). No differences in GFP expression in DA and DB neurons were seen between wild-type and *jd1500* animals (Figure 5).

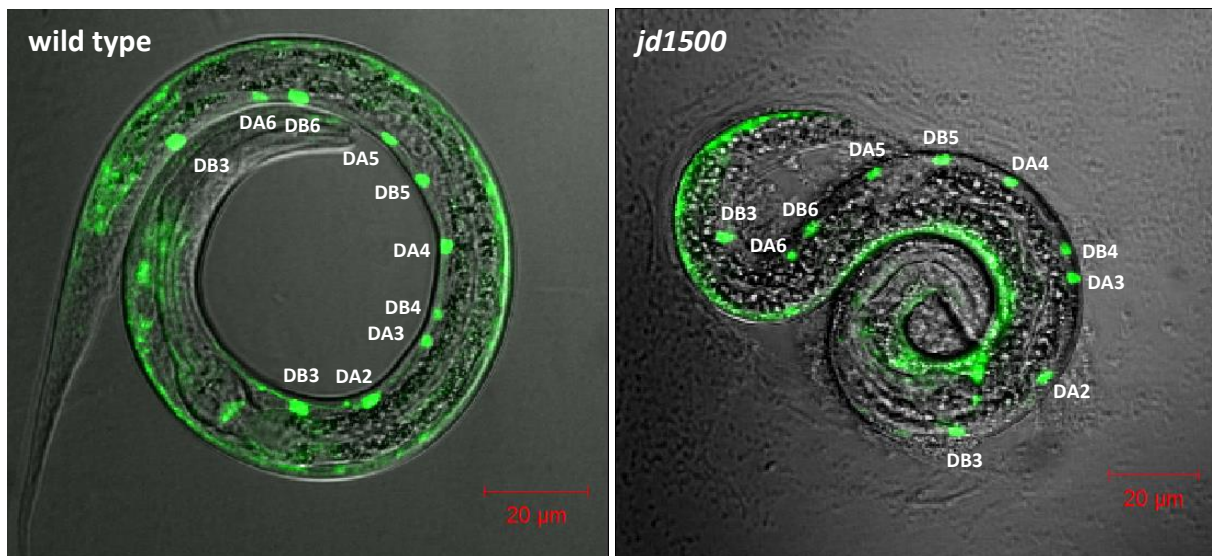


Figure 5. Fluorescent expression of *punc-129::gfp* in DA and DB motor neurons. (n=10).

3.3 Locomotion analysis of double mutants

Cellular networks composed of interneurons and excitatory motor neurons are dedicated to forward and backward movement. Both networks converge on the cross-inhibitory network formed by the D-type motor neurons and the muscles. A number of genes affect specific classes of neurons in these pathways. To gain more insight into the cellular pathways disrupted by *jd1500*, we constructed double mutants with *jd1500* and mutants known to affect specific classes of motor neurons. We then compared the phenotypes of each double mutant with the phenotypes of single mutants. The locomotion patterns of the following double mutants were observed: *unc-4/jd1500*, *unc-25/jd1500*, and *unc-42/jd1500*.

In animals with a mutation in *unc-4*, the AVB and PVC interneurons that normally innervate the VB neurons also innervate the VA motor neurons. The AVA, AVD, and AVE interneurons that normally innervate the VA neurons no longer innervate the VA neurons, leading to disrupted backward locomotion due to a reduction in excitation and contraction of ventral muscle (Miller et al., 1992). In addition, *unc-4* mutants show a subtle forward defect due to excessive contraction of ventral muscles. The *unc-4/jd1500* double mutant was severely uncoordinated to the point of paralysis during both forward and backward locomotion. We interpret the increase in the severity of the forward locomotion defect as an additive effect that suggests that *jd1500* likely acts independently of the *unc-4* gene program and the VB cellular network.

Animals with a mutation in *unc-25* demonstrate disrupted release of GABA. The absence of inhibition onto dorsal and ventral muscle leads to the shrinking of the body both dorsally and ventrally as the normal phase relationship between dorsal and ventral contraction is lost (McIntire et al., 1993). The *unc-25/jd1500* double mutant showed the coiler phenotype when moving forward and the shrinker phenotype when attempting to move backward. The additive phenotype observed suggested that

jd1500 likely acts independently of the *unc-25* gene program expressed in the DD and VD cellular networks.

In *unc-42* mutants, cell-surface receptors required for AVA, AVD, and AVE interneuron specification are not expressed, leading to more abrupt bending of the body when moving backward (known as “kinker” phenotype) (Baran et al., 1999). The *unc-42/ jd1500* double mutant showed the coiler phenotype when moving forward and the kinker phenotype when moving backward. Since an additive phenotype was observed, this suggests that *jd1500* likely acts independently of the *unc-42* gene program required for AVA, AVD, and AVE interneuron specification.

3.4 Aldicarb-sensitivity assay

Aldicarb is an acetylcholinesterase inhibitor that causes the accumulation of acetylcholine at the synaptic cleft and leads to the paralysis of wild-type nematodes (Johnson & Russell, 1983). To test if *jd1500* mutants have defects or enhancements in synaptic transmission, *jd1500* mutants were analyzed for any resistance or sensitivity to aldicarb. The N2 strain was used as the negative control and *dgk-1* (strong hypersensitivity), and *snt-1* (strong resistance) were used as positive controls as recommended by Mahoney et al. (2006). The *jd1500* mutant showed statistically significant differences in ratio of worms moving at 120, 150, and 180 minutes compared to *snt-1*, and 60 and 90 minutes compared to *dgk-1*. The *jd1500* mutants had a mild hypersensitivity to aldicarb compared to wild-type animals (Figure 6).

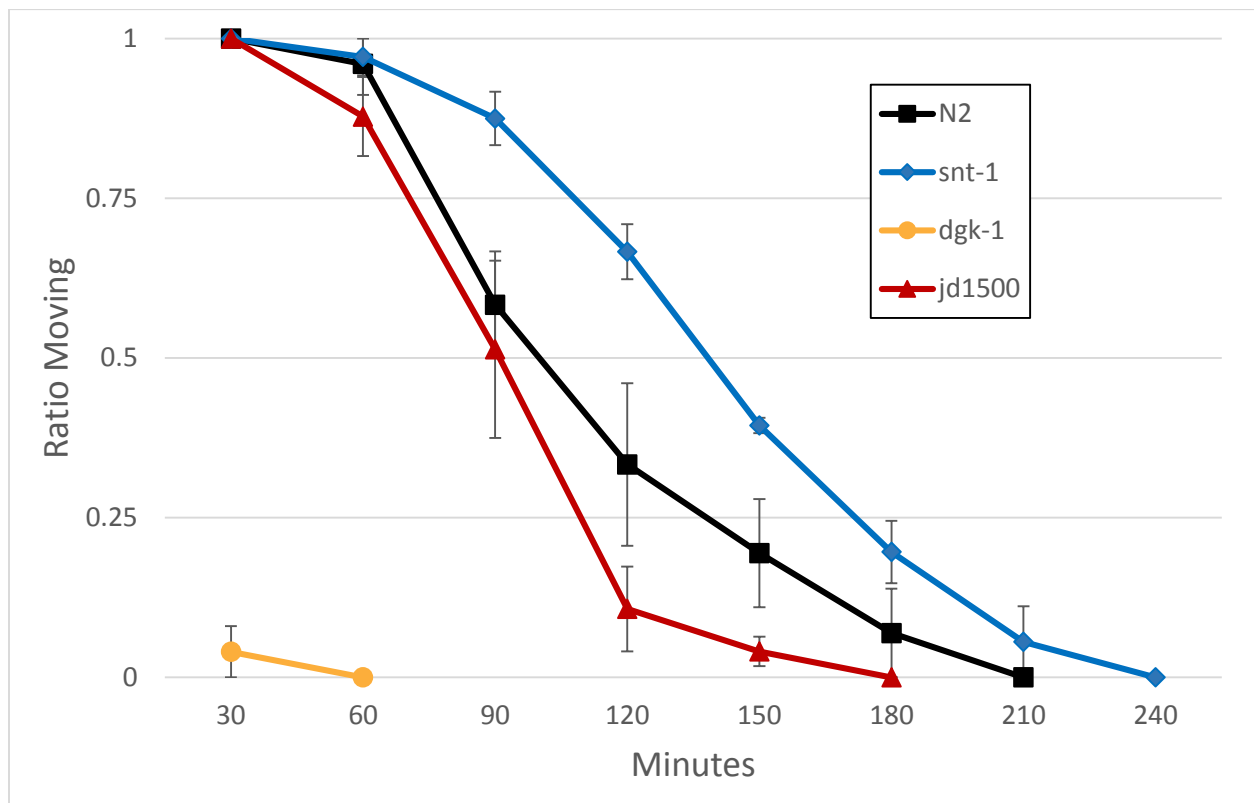


Figure 6. Aldicarb sensitivity assay of *jd1500* mutant.

Error bars represent SEM. Significance was calculated using Student's t-test (n=25).

3.5 Genetic location and identification

After determining that the mutation is X-linked, we performed three-point mapping to identify the approximate genetic and genomic location of the mutation. We used *lon-2* (X: -6.74) and *dpy-6* (X: 0.00) as our genetic markers. The following double mutants were constructed: *lon-2/jd1500* and *dpy-6/jd1500*. The *lon-2/jd1500* mutant was then mated with wild-type animals, and heterozygous hermaphrodites from the progeny were isolated to generate self-progeny. Each worm in the self-progeny was screened for the following phenotypes: wild type, long coiler, coiler-non-long, and long-non-coiler. The mapping process was repeated for *dpy-6/jd1500*, and the following phenotypes were screened for from the self-progeny of the heterozygous hermaphrodites: wild type, dumpy coiler, coiler-non-dumpy, and dumpy-non-coiler. The recombination frequency between the two genetic loci for each genetic marker was then calculated (Table 2). We determined the locus of *jd1500* to be on the upper arm of the X chromosome, between -9.42 and -11.73 centimorgans (Figure 7).

Table 2. Recombination frequency of *jd1500* with *dpy-6* and *lon-2* on the X chromosome.

		Phenotype				
Genetic marker	Genetic locus	Wild type	Long coiler	Coiler-non-long	Long-non-coiler	Recombination Frequency
<i>lon-2</i>	-6.74	1193	370	22	21	2.68%
		Wild type	Dumpy coiler	Coiler-non-dumpy	Dumpy-non-coiler	
<i>dpy-6</i>	0.00	664	171	52	59	11.73%

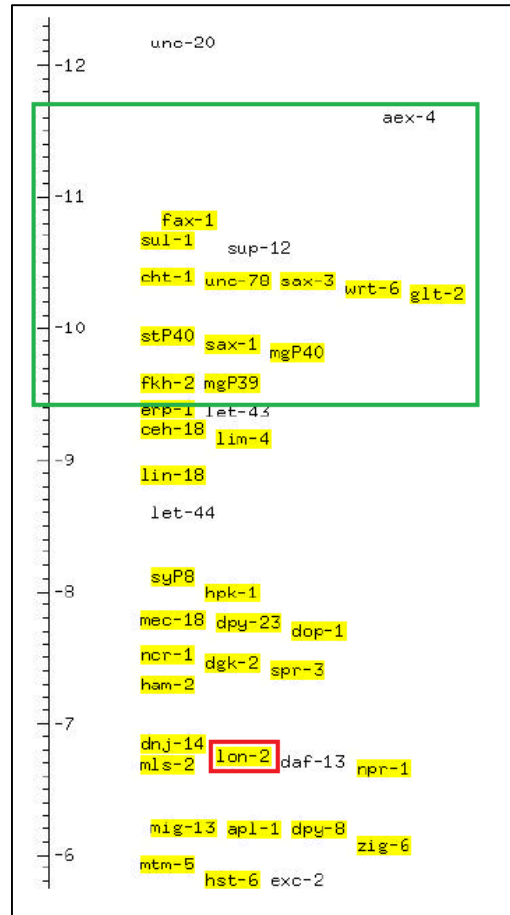


Figure 7. Predicted locus of *jd1500*.

The green box indicates the locus where *jd1500* is predicted to be from three-point mapping. *dpy-6* (X: 0.00) is not shown on map. Modified from Chen et al. 2003.

We performed deficiency mapping to confirm the three-point mapping results and determine whether the mutation is a hypomorphic or null allele. First, *jd1500* males were mated with hermaphrodites of the deficiency strain (+/*szT1* [*lon-2*]; *syDf1*/*szT1*) containing a deleted region from -11.73 to -4.24 centimorgans on the X chromosome. The progeny from the mating yielded hermaphrodites that displayed either wild-type or uncoordinated locomotive phenotypes. The uncoordinated hermaphrodites in the progeny displayed coiling when moving forward and wild-type locomotion when moving backward. The severity of the uncoordinated phenotype was indistinguishable from the phenotype observed in *jd1500* mutants.

Forward mutagenesis with chemical and physical mutagens has been performed frequently by various labs over past years. It is possible that the mutation in *jd1500* affects a gene that has already been identified and mapped. If so, mutants exhibiting a ventral bias during forward locomotion in this region of the X chromosome have not been reported. By searching the WormBase database (www.wormbase.org), we found six mutant strains which show a coiler phenotype: *sax-3*, *unc-1*, *unc-3*, *unc-10*, *unc-20*, and *unc-125*. Based on three-point mapping results with *lon-2* and *dpy-6*, we narrowed down the predicted location of *jd1500* and searched for candidate genes in the region between -9.42 and -11.73 centimorgans of the X chromosome (Table 3). The criteria used for candidate selection was based on the locomotive phenotype of *jd1500* as well as gene involvement in cell specification or development. We performed complementation tests with *fax-1*, *unc-78*, *sax-3*, *unc-2*, *unc-20*, *fkx-2*, *dop-1* to determine if the *jd1500* mutation was allelic to any of these genes. After mating *jd1500* males with each candidate strain, the progeny of each mating yielded hermaphrodites that displayed wild-type locomotion. As a positive control, *jd1500* males were mated with *jd1500* hermaphrodites. The progeny of the mating yielded hermaphrodites and males showing the coiler phenotype when moving forward.

Table 3. Candidate gene loci on the X chromosome that could be allelic with *jd1500*.

Gene and mutant phenotype descriptions are from www.wormbase.org. Mutant phenotype descriptions are from Riddle et al., 1997.

Gene	Genetic Position	Description	Mutant Phenotype
<i>fax-1</i>	-10.75	<i>fax-1</i> encodes a conserved nuclear receptor that contains two C4-type zinc fingers and is orthologous to the vertebrate photoreceptor-specific nuclear receptor PNR (OMIM:604485, mutated in enhanced S-cone syndrome and retinitis pigmentosa); <i>fax-1</i> is required for normal locomotion and neuron fate specification, including specification of the AVA, AVE, and AVK interneurons and proper axon pathfinding of the AVK, HSNL, and PVQL axons.	omega turns variant; habituation variant; plate tap reflex variant
<i>unc-78</i>	-10.34	<i>unc-78</i> encodes a WD40 repeat-containing protein homologous to actin-interacting protein (AIP1) that regulates the ordered assembly of actin and cofilin in myofibrils; homozygous <i>unc-78</i> mutant animals are viable but exhibit disorganization of actin filaments in the body wall muscle.	aldicarb resistant, slow, abnormal body muscle birefringence; abnormal muscle ultrastructure with large aggregates of thin filaments
<i>sax-3</i>	-10.34	<i>sax-3</i> encodes, by alternative splicing, two isoforms of an ortholog of <i>Drosophila</i> ROUNDABOUT, ROBO3, and LEAK, and of human ROBO1, ROBO2, and ROBO3. In larval hermaphrodites, SAX-3 is required to confine migrating sex myoblasts to the ventral muscle quadrants during their migration through the body and for multiple aspects of sensory, motor, and interneuron axon guidance.	mild coiler (certain alleles)
<i>unc-2</i>	-13.79	<i>unc-2</i> encodes a calcium channel alpha subunit similar to the human P/Q-type calcium channel, CACNA1A; <i>unc-2</i> is required for desensitization to dopamine, normal movement, normally low sensitivity of whole animals to serotonin, and neuronal migrations; <i>unc-2</i> interacts with the TGF beta pathway to regulate movement, and maintain normal serotonin levels. UNC-2 is expressed primarily in motor neurons, several sensory neurons, and the HSN and VC neurons controlling egg-laying.	weak kinker, sluggish, thin, aldicarb resistant
<i>ceh-30</i>	-12.42	<i>ceh-30</i> encodes a homeodomain protein most similar to <i>Drosophila</i> and mammalian BarH1 (OMIM:605211) which function in neuronal cell fate determination; CEH-30 functions as a key regulator of sex-specific apoptosis	cell death sexually dimorphic variant

<i>spc-1</i>	-12.14	<i>spc-1</i> encodes the <i>C. elegans</i> alpha spectrin ortholog; during development, <i>spc-1</i> activity is required for body morphogenesis, formation of body wall muscles, locomotion, and larval development.	aldicarb resistant, dumpy, embryonic lethal, locomotion variant, mitosis variant
<i>unc-20</i>	-11.61	<i>unc-20</i> is involved in locomotion.	temperature sensitive; at 25°C, severe kinker, some coiling; active, healthy; some head muscle contraction; defects in longitudinal axon elongation in VNC
<i>wrt-6</i>	-10.31	<i>wrt-6</i> encodes a hedgehog-like protein, with (from N- to C-terminus) a signal sequence, a Wart domain, an short region of low-complexity sequence, and a Hint/Hog domain. WRT-6 is also required for normal growth to full size and locomotion; all of these requirements may reflect common defects in cholesterol-dependent hedgehog-like signaling or in vesicle trafficking.	locomotion variant, small, molt defect, body vacuole
<i>sax-1</i>	-9.91	The <i>sax-1</i> gene encodes a homolog of Ndr kinase that regulates neuronal cell shape and neurite initiation.	axon outgrowth variant
<i>fkh-2</i>	-9.5	<i>fkh-2</i> encodes one of 15 <i>C. elegans</i> forkhead transcription factors. <i>fkh-2</i> is required, along with <i>ceh-37</i> , for proper development of the AWB chemosensory neurons; specifically, <i>fkh-2</i> is required for development of AWB-specific ciliary and dendritic structures and for positive regulation of expression of genes such as <i>str-1</i> , <i>odr-1</i> , and <i>kap-1</i>	embryonic lethal; sluggish
<i>dop-1</i>	-7.65	<i>dop-1</i> encodes a D1-like dopamine receptor; <i>dop-1</i> is required cell autonomously in the touch neurons for modulation of mechanosensory behaviors such as tap habituation; in addition, <i>dop-1</i> is required for regulation of locomotion via antagonism of the DOP-3 D2-like dopamine receptor in cholinergic neurons; DOP-1 is expressed in several different types of cells including mechanosensory neurons, cholinergic motor neurons, interneurons, excretory gland cells, head muscles, and neuronal support cells;	backward locomotion variant, forward locomotion decreased, NaCl chemotaxis variant, thin, touch resistant

4. DISCUSSION

Describing how cellular networks interact to give rise to complex behaviors continues to be a challenge in biological research. Locomotion in *C. elegans* has become an important model for the investigation of gene networks that create and maintain cellular networks that are capable of generating coordinated motor outcomes. *C. elegans* allows the exploitation of the well described connectivity diagrams of the nerves and muscles constructed by White et al. (1986) with the ability to obtain mutants that disrupt these networks. A dedicated network of identified interneurons, motor neurons, and muscles generates forward locomotion. By disrupting the proper function of this cellular networks via genetic perturbations, we can begin to understand the gene regulatory networks that assemble and maintain this cellular networks.

In order to investigate how forward locomotion is resistant to genetic mutations, we isolated and characterized an uncoordinated mutant from a forward genetic screen. Based on our three-point mapping, we determined that the locus of *jd1500* was on the upper arm of the X chromosome, between -9.42 and -11.73 centimorgans. We confirmed the region by mating *jd1500* into a deficiency strain (+/szT1 [lon-2]; syDf1/szT1) containing a deletion from -11.73 to -4.24 centimorgans on the X chromosome and finding coiler hermaphrodites in the progeny. The severity of the uncoordinated phenotype was identical to the phenotype observed in *jd1500* mutants, and is likely representative of the phenotype of a null allele.

Complementation tests with *jd1500* and known mutations in the region of interest on the X chromosome (*fax-1*, *unc-78*, *sax-3*, *unc-2*, *unc-20*, *fkh-2*, and *dop-1*) revealed that *jd1500* was not an allele of any of these genes. Four other candidate genes, *ceh-30*, *spc-1*, *wrt-6*, and *sax-1*, were found in the predicted region of the X chromosome, but mutant strains were not available from the *Caenorhabditis* Genetics Center. However, the description of the mutant phenotypes for these genes does not resemble that of *jd1500*. Based upon these results, we conclude that *jd1500* is likely to be a

mutation not previously characterized. In order to confirm this and aid to further characterize the cellular network affected by the mutation, whole-genome next-generation sequencing can be done to efficiently identify the locus of the molecular lesion (Hobert, 2010). By comparing the genome of the mutant to a non-mutagenized genome, the altered sequence could be found within the region predicted from three-point mapping.

Since *jd1500* is uncoordinated when moving forward but moves backward well, the ventral and dorsal body wall muscles are not likely to be defective in the mutant. This is because the same set of muscles are used for both forward and backward locomotion (White et al., 1986). This led us to investigate the nervous system for defects. The uncoordinated behavior was observed in the early L1 stage, which suggested that the neurons born post-embryonically are not responsible for the phenotype. Because *jd1500* often shows either a ventral bias or less often a dorsal bias when moving forward, we predicted that the embryonic, cholinergic DB motor neurons as likely cellular targets of the mutation. We looked at the GFP expression of *unc-129* in the DA and DB neurons using the *punc-129::gfp* reporter and did not see a difference in neurons present. Since the neurons are present, one reason for the phenotype could be disrupted synaptic transmission.

In order to understand the cellular pathways disrupted by *jd1500*, we generated double mutants with *jd1500* and mutants known to affect specific classes of motor neurons. We compared the uncoordinated phenotypes of mutants known to disrupt cellular networks responsible for aspects of locomotion. The general reasoning is that if two mutations affect the same phenotype, then the double mutant will be difficult to distinguish from the two individual mutants. As we saw additive phenotypes for doubles affecting the DD and VD motor neurons, we conclude that *jd1500* likely does not directly affect the cross-inhibitory function of the D-type neurons. We also observed additive phenotypes for doubles affecting AVA, AVD, and AVE specification, and conclude that *jd1500* does not directly affect the *unc-42* gene program required for the specification of interneurons that regulate backward locomotion.

The *unc-4; jd1500* double mutants were severely uncoordinated to the point of paralysis during both forward and backward locomotion. Mutations in *unc-4* mutants show a greater defect on backward than forward locomotion, but careful observation reveals a subtle ventral bias due to the additional excitatory input onto ventral muscles. It is possible that the mutation of *jd1500* is causing a more severe uncoordinated phenotype affecting forward locomotion in the double mutants. We interpret the *unc-4; jd1500* double mutant phenotype as an additive effect, and conclude that *jd1500* likely does not directly affect the VA and VB cellular network. An additive effect (the increased severity in forward uncoordinated locomotion) may be due to the known effect on the VB neurons and the suspected effect on the DB neurons.

Mutants that cannot synthesize or release acetylcholine, *cha-1* and *unc-17* respectively, demonstrate a coil-like shape when attempting to move (Rand & Russell, 1984, Alfonso et al., 1993). For this reason, *cha-1* and *unc-17* mutants could not be used for double mutant locomotion analysis, as these mutants show a similar locomotive phenotype to *jd1500* mutants. Instead we used the cholinesterase inhibitor aldicarb to determine if *jd1500* had altered synaptic transmission. Our results show a trend that *jd1500* has a mild hypersensitivity to aldicarb. Generally, mutant strains that produce excess acetylcholine will accumulate acetylcholine at a quicker rate and will be hypersensitive to aldicarb. However, we cannot make this conclusion as we did not see a significant difference between *jd1500* and wild type in the ratio of moving worms at any of the time points. We can conclude that the *jd1500* phenotype is not likely due to a defect in the postsynaptic acetylcholine receptors, as the *jd1500* mutants become paralyzed at a similar rate compared to wild-type animals in response to aldicarb exposure.

If chemical synaptic transmission is not altered in *jd1500* mutants, one possible explanation for the uncoordinated phenotype is a defect in gap junctions. The B-type motor neurons form gap junctions with neighboring motor neurons of their class and also with the AVB interneurons (White et al., 1986).

The DB neurons express *inx-3* and *unc-9*, which encode innexin proteins required for gap junction formation (Starich et al., 2001; Barnes & Hekimi, 1997). A double mutant analysis with *inx-3* and *unc-9* could give insight into the genetic pathway that *jd1500* is involved in.

In order to prevent hypercontraction of the dorsal or ventral body muscles during sinusoidal locomotion, negative feedback loops must be used to signal adjacent muscle cells to contract at certain set points. Li et al. (2006) found that the DVA interneuron acts as a proprioception receptor that detects the stretch of muscles and suppresses excess body bending. However, ablations of DVA show that it is not required for the anterior to posterior propagation of muscle contractions observed in forward locomotion (Wen et al., 2012). The DB and VB neurons both have long posterior neurites that extend further than their neuromuscular junctions and do not make synapses (White et al., 1986). These neurites were initially proposed to act as proprioceptive receptors that regulate the action of adjacent motor neurons in response to the contraction of body wall muscles, leading to the propagation of the sinusoidal locomotion pattern (L. Byerly & R.L. Russell, personal communications, cited by White et al. 1986; Riddle et al., 1997).

Wen et al. (2012) used optogenetics and calcium imaging to manipulate locomotive circuit activity and show that proprioceptive coupling between the B-type motor neurons regulates the sinusoidal wave propagation used in forward locomotion. Furthermore, Qi et al. (2013) have found that expressing a gain-of-function mutation in *acr-2* in either the VB or DB neurons leads to a disruption of sinusoidal movement and arrhythmic convulsions. ACR-2 is an acetylcholine receptor located on the soma and dendrites of the cholinergic motor neurons (Jospin et al., 2009; Qi et al., 2013). This further supports that the B-type neurons are involved in the propagation of the sinusoidal locomotive pattern. If negative feedback loops are disrupted, then muscle cells continue to contract beyond their set points. This is a potential explanation for why *jd1500* mutants coil with a strong bias when moving forward, as

adjacent body wall muscle cells are not coupled by the B-type neurons to contract at their appropriate magnitudes.

A potential explanation for why *C. elegans* spend more time moving forward rather than backward is because most neurons of the worm are located in ganglia of the head, 68 of which are sensory neurons (White et al., 1986). However, why the neuromuscular networks responsible for forward locomotion are robust against genetic perturbations remains unclear. By using a forward genetic approach in *C. elegans*, we can dissect the cellular networks that regulate proper locomotive patterns. Here we describe a mutation that disrupts forward locomotion, but not backward locomotion. Only a few genetic mutations affect forward locomotion without disrupting backward locomotion. In *unc-23* mutants, dystrophy of head muscles prevents proper attachment of musculature to hypodermis, leading the head to bend dorsally or ventrally (Waterston et al., 1980). These mutants have disrupted forward locomotion due to inability to control head movement. Animals with mutations in *unc-7* and *unc-9* have a disruption in innexin production required for gap junction formation, and demonstrate the kinker phenotype when moving forward, but move backward well (Starich et al., 1993, Barnes & Hekimi, 1997). It is unclear why mutations in *unc-7* and *unc-9* more severely affect forward locomotion, as these genes are expressed in various interneurons and motor neurons of the ventral nerve cord. By further characterizing mutants that demonstrate locomotive biases like the *jd1500* mutant, we can learn more about the dedicated neural networks responsible for forward and backward locomotion.

Understanding why some mutants have particular biases in locomotion in the nematode can give insight into how certain heart conditions may arise due to disruptions in the coordination of motor programs. A variety of heart conditions exist due to arrhythmic contractions of muscles in the heart. For example, cardiac arrhythmia describes an abnormal rhythm of heart contractions, which can lead to heart failure, and in some cases, cardiac arrest. A few genes have been identified that are responsible for inherited arrhythmia syndromes. However, the diagnostic yield of genetic testing for these genes

varies widely (Sturm & Hershberger, 2013). The research in this thesis investigates a gene that when mutated appears to have removed the set points governing the minimum and maximum lengths of muscle during the contraction and relaxation cycle. Similar disruptions in cardiac cellular networks could lead to abnormal rhythms. By studying the genetic programs that assemble and maintain cellular networks, as well as circuit components that provide compensatory feedback to ensure function within appropriate domains in the nematode, we can better understand cellular networks that generate complex motor programs such as the beat of the heart.

REFERENCES

1. Alfonso, A., Grundahl, K., Duerr, J. S., H-P, H., & Rand, J. B. (1993). The *Caenorhabditis elegans* *unc-17* gene: a putative vesicular acetylcholine transporter. *Science*, 261, 617-9.
2. Altun, Z.F. and Hall, D.H. (2011). Nervous system, general description. In *WormAtlas*. doi:10.3908/wormatlas.1.18
3. Akay, T., Tourtellotte, W. G., Arber, S., & Jessell, T. M. (2014). Degradation of mouse locomotor pattern in the absence of proprioceptive sensory feedback. *Proceedings of the National Academy of Sciences of the United States of America*, 111(47), 16877–16882. <http://doi.org/10.1073/pnas.1419045111>
4. Azevedo, F. A.C., Carvalho, L. R.B., Grinberg, L. T., Farfel, J. M., Ferretti, R. E.L., Leite, R. E.P., Filho, W. J., Lent, R. and Herculano-Houzel, S. (2009), Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *J. Comp. Neurol.*, 513, 532–541. doi: 10.1002/cne.21974
5. Baran, R., Aronoff, R., and Garriga, G. (1999). The *C. elegans* homeodomain gene *unc-42* regulates chemosensory and glutamate receptor expression. *Development*, 126, 2241-51.
6. Barnes TM, Hekimi S. (1997). The *Caenorhabditis elegans* avermectin resistance and anesthetic response gene *unc-9* encodes a member of a protein family implicated in electrical coupling of excitable cells. *J Neurochem*, 69, 2251–2260.
7. Brenner S. (1973). The genetics of behavior. *Br. Med. Bull*, 29, 269–271.
8. Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics*. 77, 71-94.
9. Brenner S. Wood W.B., editor. (1988). The nematode *Caenorhabditis elegans*. *Cold Spring Harbor, New York: Cold Spring Harbor Laboratory*. Foreword.
10. Brown TG. (1911). The Intrinsic Factors in the Act of Progression in the Mammal. *P Roy Soc Lond B Bio.*, 84,308–319.
11. Bruneau, B. G. (2013). Signaling and transcriptional networks in heart development and regeneration. *Cold Spring Harb. Perspect. Biol.* 5, a008292.
12. Chalfie M., Sulston J.E., White J.G., Southgate E., Thomson J.N., Brenner S. (1985). The Neural Circuit for Touch Sensitivity in *Caenorhabditis-Elegans*. *Journal of Neuroscience*, 5, 956–964.
13. Chen, C-K., Bradnam K., Durbin R., Hodgkin J. (2003). Genetic map of *Caenorhabditis elegans*. *Caenorhabditis Genetics Center*, St. Paul, Minnesota.
14. Chou, H. T., Vazquez, R. G., Wang, K., Campbell, R., Milledge, G. Z., Walthall, W. W., and Johnson, C. M. (2015). HES-Mediated Repression of Pten in *Caenorhabditis elegans*. *G3*:

Genes/Genomes/Genetics, 5(12), 2619–2628. <http://doi.org/10.1534/g3.115.019463>

15. Colavita, A. and Culotti, J. G. (1998). Suppressors of ectopic UNC-5 growth cone steering identify eight genes involved in axon guidance in *Caenorhabditis elegans*. *Dev Biol*, 194, 72-85. doi:10.1006/dbio.1997.8790
16. Colavita A., Krishna S., Zheng H., Padgett R. W., Culotti J. G. (1998). Pioneer axon guidance by UNC-129, a *C. elegans* TGF-beta. *Science*, 281, 706–709.
17. Esmaili, B., Ross, J. M., Neades, C., Miller, D. M., and Ahringer, J. (2002). The *C. elegans* even-skipped homologue, *vab-7*, specifies DB motoneurone identity and axon trajectory. *Development*, 129, 853-62.
18. Gray J, Lissmann HW. (1964). The locomotion of nematodes. *Journal of Experimental Biology*, 41, 135–154.
19. Hobert, O. (2010). The Impact of Whole Genome Sequencing on Model System Genetics: Get Ready for the Ride. *Genetics*, 184(2), 317–319. <http://doi.org/10.1534/genetics.109.112938>
20. Jin, Y. S., Hoskins, R., and Horvitz, H. R. (1994). Control of type-D GABAergic neuron differentiation by *C. elegans* UNC-30 homeodomain protein. *Nature*, 372, 780-3. doi:10.1038/372780a0
21. Johnson, C.D. and Russell, R.L. (1983). Multiple molecular forms of acetylcholinesterase in the nematode *Caenorhabditis elegans*. *J. Neurochem*, 41, 30–46.
22. Jorgensen E.M. and Mango S.E. (2002). The art and design of genetic screens: *Caenorhabditis elegans*. *Nat Rev Genet*, 3,356–369
23. Jospin M., Qi Y.B., Stawicki T.M., Boulin T., Schuske K.R., Horvitz H.R., Bessereau J.L., Jorgensen E.M., Jin Y. (2009). A neuronal acetylcholine receptor regulates the balance of muscle excitation and inhibition in *Caenorhabditis elegans*. *PLoS biology*, 7:e1000265
24. Kratsios, P., Stolfi, A., Levine, M., and Hobert, O. (2012). Coordinated regulation of cholinergic motor neuron traits through a conserved terminal selector gene. *Nature Neuroscience*, 15(2), 205–214.
25. Lewis J.A., Wu C.H., Berg H., Levine J.H. (1980). The genetics of levamisole resistance in the nematode *Caenorhabditis elegans*. *Genetics*, 95, 905-928.
26. Levy M.N. (1972). Sympathetic–parasympathetic interactions in the heart. *Circ Res* 29, 437
27. Li, W., Feng, Z., Sternberg, P. W., & Shawn Xu, X. Z. (2006). A *C. elegans* stretch receptor neuron revealed by a mechanosensitive TRP channel homologue. *Nature*, 440(7084), 684–687.

<http://doi.org/10.1038/nature04538>

28. Mahoney T.R., Luo S., Nonet M.L. (2006). Analysis of synaptic transmission in *Caenorhabditis elegans* using an aldicarb-sensitivity assay. *Nat Protoc.*, 1(4), 1772–7. doi: 10.1038/nprot.2006.281.
29. McIntire S.L., Jorgensen E., Kaplan J., Horvitz H.R. (1993). The GABAergic nervous system of *Caenorhabditis elegans*. *Nature*, 364, 337-341.
30. Qi, Y. and Ge, H. (2006). Modularity and Dynamics of Cellular Networks. *PLoS Computational Biology*, 2(12), e174. <http://doi.org/10.1371/journal.pcbi.0020174>
31. Qi, Y. B., Po, M. D., Mac, P., Kawano, T., Jorgensen, E. M., Zhen, M., & Jin, Y. (2013). Hyperactivation of B-type motor neurons results in aberrant synchrony of the *C. elegans* motor circuit. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, 33(12), 5319–5325. <http://doi.org/10.1523/JNEUROSCI.4017-12.2013>
32. Rand, J. B., & Russell, R. L. (1984). Choline acetyltransferase-deficient mutants of the nematode *Caenorhabditis elegans*. *Genetics*, 106, 227-48.
33. Richmond J.E., Jorgensen E.M. (1999) One GABA and two acetylcholine receptors function at the *C. elegans* neuromuscular junction. *Nat Neurosci*, 2, 791-797.
34. Riddle D.L., Blumenthal T., Meyer B.J., et al., editors. (1997) *C. elegans* II. 2nd edition. Cold Spring Harbor (NY): *Cold Spring Harbor Laboratory Press*. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK19989/>
35. Rossignol, S., Dubuc, R., Gossard, J. (2006). Dynamic Sensorimotor Interactions in Locomotion. *Physiological Reviews*, 86 (1), 89-154.
36. Shan G., Kim K., Li C., Walthall W.W. (2005). Convergent genetic programs regulate related motor neuron classes similarities and differences between in *Caenorhabditis elegans*. *Dev Biol*, 280, 494-503. [10.1016/j.ydbio.2005.01.032](https://doi.org/10.1016/j.ydbio.2005.01.032)
37. Silverman, M. E., and Hollman, A. (2007). Discovery of the sinus node by Keith and Flack: on the centennial of their 1907 publication. *Heart*, 93(10), 1184–1187. <http://doi.org/10.1136/hrt.2006.105049>
38. Starich T.A., Herman R.K., Shaw J.E. (1993). Molecular and genetic analysis of *unc-7*, a *Caenorhabditis elegans* gene required for coordinated locomotion. *Genetics*, 133, 527–541.
39. Starich, T., Sheehan, M., Jadrlich, J., and Shaw, J. (2001). Innexins in *C. elegans*. *Cell Commun Adhes*, 8, 311-4. doi:10.3109/15419060109080744
40. Sturm A.C., Hershberger R.E. (2013). Genetic testing in cardiovascular medicine: current landscape and future horizons. *Curr Opin Cardiol.*, 28, 317–325.

41. Sulston J.E. & Horvitz H.R. (1977) Post-embryonic cell lineages of the nematode *Caenorhabditis elegans*. *Dev. Biol.*, *56*, 110–156.
42. Sulston J.E., Schierenberg E., White J.G., Thomson J.N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.*, *100*, 64–119.
43. The *C. elegans* Sequencing Consortium (1998). Genome Sequence of the Nematode *C. elegans* : A Platform for Investigating Biology. *Science*, *282*, 2012–2019.
<http://doi.org/10.1126/science.282.5396.2012>
44. Walthall W.W. & Plunkett J.A. (1995). Genetic transformation of the synaptic pattern of a motoneuron class in *Caenorhabditis elegans*. *J Neurosci*, *15*, 1035-1043
45. Waterston, R. H., Thomson, J. N., & Brenner, S. (1980). Mutants with altered muscle structure of *Caenorhabditis elegans*. *Dev Biol*, *77*, 271-302. doi:10.1016/0012-1606(80)90475-3
46. Wen, Q., Po, M., Hulme, E., Chen, S., Liu, X., Kwok, S. W., ... Samuel, A. D. T. (2012). Proprioceptive coupling within motor neurons drives *C. elegans* forward locomotion. *Neuron*, *76*(4), 750–761.
<http://doi.org/10.1016/j.neuron.2012.08.039>
47. White J.G., Southgate, E., Thomson, J.N. and Brenner, S. 1976. The structure of the ventral nerve cord of *Caenorhabditis elegans*. *Philos. Trans. R. Soc. Lond. Series B. Biol. Sci.*, *275B*, 327-348.
48. White, J.G., Southgate, E., Thomson, J.N. and Brenner, S. 1986. The structure of the nervous system of the nematode *C. elegans*. *Philos. Trans. R. Soc. Lond. Series B. Biol. Sci.*, *314*, 1-340.
49. Winnier, A. R., Meir, J. Y.-J., Ross, J. M., Tavernarakis, N., Driscoll, M., Ishihara, T., ... Miller, D. M. (1999). UNC-4/UNC-37-dependent repression of motor neuron-specific genes controls synaptic choice in *Caenorhabditis elegans*. *Genes & Development*, *13*(21), 2774–2786.
50. Wolkow, C.A. and Hall, D.H. (2015). Introduction to the Dauer Larva, Overview. In *WormAtlas*. doi:10.3908/wormatlas
51. WormBase web site (2016). Retrieved from <http://www.wormbase.org>, release WS253.
52. Von Stetina, S.E., Fox, R.M., Watkins, K.L., Starich, T.A., Shaw, J.E. and Miller, D.M., 3rd. (2007). UNC-4 represses CEH-12/HB9 to specify synaptic inputs to VA motor neurons in *C. elegans*. *Genes Dev.*, *21*, 332-346.
53. Zhou, H. M., & Walthall, W. W. (1998). UNC-55, an orphan nuclear hormone receptor, orchestrates synaptic specificity among two classes of motor neurons in *Caenorhabditis elegans*. *J Neurosci*, *18*, 10438-44.