Supplementary information for

"Systems level circuit model of *C. elegans* undulatory locomotion: mathematical modeling and molecular genetics"

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1. **Supplementary Figure.**

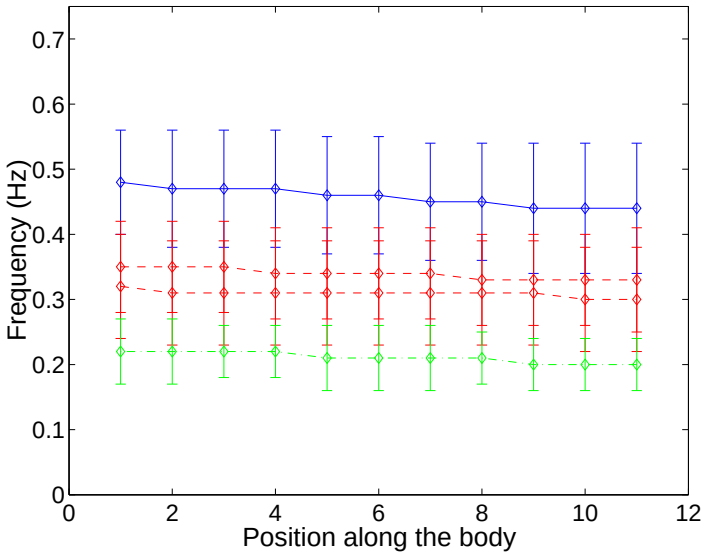

Figure S1. Experimental dependence of the undulatory frequency on calcium signaling in muscle cells. Mutants with defective calcium signaling *egl-19(n582); unc-119::egl-19::yfp* (these mutants are defective in neuronal and muscle calcium signaling but rescued in neurons) (the dashed red lines represent two different transgenic lines) and *unc-68(r1168)* (dashed-dotted green lines) have significantly lower frequencies than wild-type (solid blue line) (non-parametric sign-test for paired average frequencies along the body, $p < 0.01$).

2. **Supplementary Tables.**

Table T1.
Distribution of the frequency of undulations and flex angle along a worm's body during forward and backward locomotion for adult wild-type (N2) *C. elegans* and mutants.

Genotype	Position					
	1 (head)	3	5	7	9	11 (tail)
C. elegans N2 (N=62)						
Frequency (Hz)						
Forward motion	0.48±0.08	0.47±0.09	0.46±0.09	0.45±0.09	0.44±0.10	0.44±0.10
Backward motion	0.41±0.14	0.33±0.16	0.30±0.13	0.32±0.15	0.28±0.14	0.31±0.16
Flex (rad)						
Forward motion	1.41±0.14	1.26±0.11	1.06±0.09	0.99±0.08	0.92±0.09	0.88±0.14
Backward motion	1.43±0.17	1.26±0.32	1.08±0.20	1.03±0.15	1.00±0.16	1.04±0.29
Neuronal Mutants:						
unc-25(e156) (N=4)						
Frequency (Hz)						
Forward motion	0.36±0.07	0.34±0.08	0.32±0.08	0.27±0.10	0.30±0.08	0.23±0.06
Backward motion	0.20±0.08	0.16±0.04	0.14±0.04	0.15±0.03	0.18±0.04	0.13±0.03
Flex (rad)						
Forward motion	1.72±0.04	1.07±0.05	0.80±0.03	0.72±0.04	0.68±0.04	0.67±0.03
Backward motion	1.51±0.14	0.99±0.10	0.78±0.11	0.83±0.18	0.89±0.22	0.95±0.31
unc-46(e177) (N=4)						
Frequency (Hz)						
Forward motion	0.29±0.03	0.24±0.04	0.21±0.05	0.16±0.03	0.18±0.06	0.16±0.07
Backward motion	0.26±0.02	0.16±0.03	0.14±0.04	0.14±0.03	0.13±0.04	0.13±0.04
Flex (rad)						
Forward motion	1.40±0.07	0.91±0.06	0.65±0.06	0.55±0.02	0.49±0.07	0.52±0.09
Backward motion	1.21±0.13	0.84±0.06	0.63±0.04	0.56±0.07	0.60±0.16	0.66±0.23
unc-18(e81);cho-1::unc-18::yfp (N=14)						
Frequency (Hz)						
Forward motion	0.20±0.08	0.15±0.07	0.12±0.06	0.10±0.05	0.09±0.05	0.09±0.04
Backward motion	0.16±0.05	0.11±0.04	0.09±0.05	0.07±0.03	0.07±0.03	0.06±0.02
Flex (rad)						
Forward motion	1.10±0.17	0.87±0.17	0.64±0.16	0.56±0.18	0.48±0.15	0.43±0.12
Backward motion	1.10±0.19	0.78±0.20	0.57±0.17	0.50±0.18	0.45±0.16	0.42±0.13

Table T1 (continued).

Genotype	Position					
	1 (head)	3	5	7	9	11 (tail)
slo-1(js118) (N=18)						
Frequency (Hz)						
Forward motion	0.38±0.07	0.35±0.07	0.33±0.07	0.32±0.07	0.30±0.07	0.29±0.08
Backward motion	0.32±0.07	0.24±0.06	0.21±0.06	0.20±0.06	0.17±0.05	0.16±0.06
Flex (rad)						
Forward motion	1.75±0.07	1.39±0.07	1.14±0.09	1.07±0.11	1.01±0.13	1.04±0.11
Backward motion	1.62±0.07	1.29±0.12	1.11±0.11	1.09±0.15	1.09±0.15	1.21±0.18
Non-neuronal Mutants:						
egl-19(n582);						
unc-119::egl-19::yfp						
Line A (N=16)						
Frequency (Hz)						
Forward motion	0.35±0.07	0.35±0.07	0.34±0.07	0.34±0.07	0.33±0.07	0.33±0.08
Backward motion	0.35±0.12	0.32±0.09	0.31±0.09	0.34±0.11	0.31±0.09	0.34±0.08
Flex (rad)						
Forward motion	1.16±0.09	1.13±0.07	1.02±0.10	0.95±0.06	0.95±0.07	0.98±0.05
Backward motion	1.15±0.09	1.16±0.13	1.04±0.15	0.99±0.15	1.02±0.17	1.20±0.24
Line B (N=16)						
Frequency (Hz)						
Forward motion	0.32±0.08	0.31±0.08	0.31±0.08	0.31±0.08	0.31±0.08	0.30±0.08
Backward motion	0.38±0.13	0.30±0.13	0.33±0.12	0.32±0.14	0.31±0.12	0.30±0.15
Flex (rad)						
Forward motion	1.23±0.11	1.24±0.09	1.12±0.11	1.03±0.09	0.98±0.06	1.02±0.09
Backward motion	1.23±0.19	1.25±0.21	1.14±0.15	1.09±0.19	1.09±0.25	1.28±0.29
unc-68(r1158) (N=16)						
Frequency (Hz)						
Forward motion	0.22±0.05	0.22±0.04	0.21±0.05	0.21±0.05	0.20±0.04	0.20±0.04
Backward motion	0.15±0.05	0.14±0.04	0.12±0.03	0.14±0.05	0.11±0.04	0.12±0.05
Flex (rad)						
Forward motion	1.53±0.10	1.26±0.14	1.09±0.15	1.01±0.13	1.02±0.14	1.07±0.15
Backward motion	1.39±0.18	1.17±0.11	1.03±0.14	0.95±0.13	0.98±0.17	1.10±0.16

Table T1 (continued).

Genotype	Position					
	1 (head)	3	5	7	9	11 (tail)
unc-54(s74) (N=5)						
Frequency (Hz)						
Forward motion	0.09±0.01	0.08±0.02	0.08±0.02	0.07±0.03	0.08±0.02	0.07±0.03
Backward motion	0.10±0.02	0.09±0.01	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.02
Flex (rad)						
Forward motion	1.63±0.27	1.38±0.24	1.13±0.29	1.10±0.26	1.17±0.23	1.20±0.27
Backward motion	1.53±0.09	1.41±0.05	1.30±0.10	1.31±0.10	1.50±0.17	1.69±0.10
unc-54(s95) (N=5)						
Frequency (Hz)						
Forward motion	0.07±0.01	0.07±0.01	0.07±0.01	0.07±0.01	0.07±0.01	0.07±0.01
Backward motion	0.06±0.01	0.06±0.01	0.06±0.00	0.06±0.01	0.06±0.00	0.06±0.01
Flex (rad)						
Forward motion	0.86±0.06	0.81±0.05	0.69±0.06	0.65±0.05	0.71±0.05	0.86±0.06
Backward motion	0.65±0.05	0.65±0.06	0.57±0.05	0.58±0.07	0.66±0.08	0.83±0.11
unc-54(st130) (N=5)						
Frequency (Hz)						
Forward motion	0.05±0.01	0.05±0.01	0.05±0.00	0.04±0.01	0.05±0.00	0.05±0.01
Backward motion	0.05±0.01	0.04±0.01	0.04±0.01	0.04±0.00	0.04±0.01	0.06±0.02
Flex (rad)						
Forward motion	0.52±0.09	0.46±0.12	0.44±0.07	0.42±0.07	0.49±0.06	0.65±0.07
Backward motion	0.45±0.07	0.41±0.09	0.40±0.06	0.39±0.05	0.46±0.05	0.64±0.06
unc-54(st132) (N=5)						
Frequency (Hz)						
Forward motion	0.16±0.02	0.16±0.03	0.15±0.03	0.15±0.03	0.15±0.03	0.15±0.03
Backward motion	0.17±0.04	0.14±0.04	0.12±0.03	0.12±0.03	0.11±0.02	0.14±0.05
Flex (rad)						
Forward motion	1.37±0.12	1.20±0.14	1.06±0.11	1.03±0.13	1.02±0.14	1.18±0.15
Backward motion	1.16±0.12	1.02±0.11	1.00±0.10	1.00±0.17	1.06±0.20	1.36±0.22
unc-54(st134) (N=5)						
Frequency (Hz)						
Forward motion	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01	0.08±0.01
Backward motion	0.08±0.02	0.08±0.02	0.07±0.02	0.08±0.02	0.07±0.02	0.10±0.02
Flex (rad)						
Forward motion	1.19±0.05	1.11±0.06	0.96±0.07	0.92±0.08	0.98±0.03	1.06±0.06
Backward motion	1.07±0.05	1.02±0.08	0.88±0.12	0.88±0.06	0.98±0.06	1.10±0.18

Table T1 (continued).

Genotype	Position					
	1 (head)	3	5	7	9	11 (tail)
unc-54(st135) (N=5)						
Frequency (Hz)						
Forward motion	0.06±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.06±0.00	0.05±0.01
Backward motion	0.06±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.06±0.01	0.06±0.01
Flex (rad)						
Forward motion	0.78±0.06	0.64±0.10	0.52±0.10	0.49±0.07	0.58±0.08	0.77±0.03
Backward motion	0.67±0.07	0.59±0.07	0.49±0.07	0.47±0.11	0.61±0.13	0.83±0.12
sqt-1(sc101) (N=13)						
Frequency (Hz)						
Forward motion	0.29±0.06	0.27±0.07	0.26±0.07	0.26±0.07	0.25±0.07	0.22±0.07
Backward motion	0.23±0.08	0.18±0.07	0.18±0.06	0.17±0.07	0.15±0.06	0.14±0.06
Flex (rad)						
Forward motion	1.40±0.08	1.14±0.13	0.93±0.14	0.83±0.08	0.76±0.09	0.72±0.13
Backward motion	1.36±0.13	1.02±0.16	0.86±0.16	0.78±0.14	0.73±0.13	0.75±0.15
sqt-1(sc103) (N=5)						
Frequency (Hz)						
Forward motion	0.29±0.07	0.28±0.10	0.27±0.10	0.26±0.10	0.25±0.10	0.22±0.09
Backward motion	0.22±0.05	0.15±0.06	0.15±0.07	0.11±0.04	0.12±0.05	0.12±0.04
Flex (rad)						
Forward motion	1.27±0.13	1.12±0.10	0.96±0.11	0.86±0.09	0.77±0.09	0.71±0.13
Backward motion	1.31±0.08	1.06±0.16	0.95±0.16	0.82±0.12	0.73±0.06	0.69±0.06
BE109 (N=9)						
Frequency (Hz)						
Forward motion	0.19±0.06	0.18±0.06	0.18±0.06	0.18±0.07	0.18±0.07	0.18±0.07
Backward motion	0.14±0.07	0.12±0.05	0.12±0.06	0.14±0.08	0.11±0.03	0.13±0.07
Flex (rad)						
Forward motion	1.48±0.16	1.62±0.17	1.54±0.20	1.46±0.21	1.40±0.30	1.16±0.33
Backward motion	1.40±0.22	1.45±0.26	1.41±0.29	1.27±0.27	1.25±0.36	1.08±0.40

All data refer to worms moving on plates with food.

Table T2.
Distribution of the frequency (Hz) of undulations along worm's body during forward locomotion for *slo-1* mutants with transgenes.

Genotype	Position					
	1 (head)	3	5	7	9	11 (tail)
slo-1(js118); Pacr-2::gfp						
(N=19)	0.43±0.07	0.38±0.09	0.32±0.11	0.30±0.12	0.28±0.12	0.25±0.13
slo-1(js118); Pacr-2::slo-1						
Line A (N=19)	0.43±0.05	0.42±0.06	0.37±0.08	0.35±0.09	0.33±0.08	0.31±0.09
Line B (N=18)	0.41±0.06	0.37±0.07	0.36±0.07	0.34±0.07	0.33±0.08	0.31±0.07
Line C (N=18)	0.37±0.07	0.33±0.09	0.31±0.09	0.29±0.09	0.28±0.09	0.27±0.09
Line D (N=17)	0.36±0.05	0.32±0.06	0.29±0.06	0.28±0.06	0.26±0.06	0.25±0.06
slo-1(js118); Pacr-2::slo-1; Punc-25::slo-1						
(N=21)	0.47±0.08	0.46±0.08	0.43±0.10	0.41±0.10	0.40±0.10	0.40±0.10
slo-1(js118); Psnb-1::slo-1						
(N=20)	0.48±0.07	0.46±0.07	0.43±0.08	0.40±0.09	0.38±0.09	0.37±0.09
slo-1(js118); Punc-25::slo-1						
Line A (N=18)	0.44±0.07	0.41±0.07	0.40±0.08	0.39±0.07	0.37±0.08	0.36±0.08
Line B (N=16)	0.46±0.07	0.42±0.09	0.41±0.10	0.40±0.10	0.38±0.10	0.37±0.10
slo-1(js118); Punc-17::slo-1						
Line A (N=16)	0.45±0.11	0.42±0.11	0.39±0.12	0.37±0.12	0.36±0.12	0.34±0.12
Line B (N=18)	0.48±0.06	0.47±0.06	0.45±0.07	0.43±0.08	0.41±0.09	0.40±0.09
Line C (N=18)	0.45±0.09	0.44±0.09	0.42±0.09	0.40±0.09	0.39±0.10	0.37±0.09
slo-1(js118); Pcho-1::slo-1						
Line A (N=18)	0.46±0.06	0.43±0.07	0.40±0.07	0.36±0.08	0.33±0.10	0.31±0.09
Line B (N=18)	0.44±0.06	0.42±0.07	0.39±0.07	0.37±0.07	0.36±0.08	0.34±0.08
Line C (N=17)	0.37±0.06	0.34±0.08	0.31±0.08	0.29±0.08	0.27±0.08	0.25±0.08

Worms *slo-1(js118); Pacr-2::slo-1; Punc-25::slo-1* and *slo-1(js118); Psnb-1::slo-1* have presumably wild-type levels of GABA and acetylcholine signaling. This is partly confirmed based on their spatial frequency patterns, which are statistically indistinguishable from the

wild-type pattern (non-parametric sign test for paired average frequencies, $p > 0.05$).

3. **Supplementary Methods**.

Molecular biology: plasmid and strain construction

a) *slo-1*: transgenic strains and plasmids.

The following strains and plasmids were a gift from Andrew Davies and Steven McIntire and the construction is described in (Davies et al, 2003):

Strains:

BZ802 *slo-1(js118); egEx25[Pacr-2::slo-I(+)Pacr::gfp]*,

BZ798 *slo-1(js118); egEx24[Pacr-2::slo-1(+); Punc-25::slo-1(+); Pacr::gfp]*,

BZ812 *slo-1(js118); egEx26[Pacr::gfp]*

and BZ416 *slo-1(js118); egEx23[Psnb-1::slo-I(+) H20::gfp]*.

Plasmids:

Punc-25::slo-1(+), *Pacr-2::slo-1(+)*, *Pacr-2::gfp* and pBK3.1 (Wang et al, 2001).

New *slo-1* transgenic strains and plasmids:

Strains: *Punc-25::slo-1(+)* was injected at 7.5 ng/μl with a coinjection marker of *Pmyo-2::gfp* at 5 ng/μl into *slo-1(js118)* animals using pBSKS as a carrier to create *slo-1(js118); Ex[Punc-25::slo-1(+) Pmyo::gfp]*. Strains carrying *Punc-17::slo-1(+)* or *Pcho-1::slo-1(+)*, were created similarly. *Pacr-2::slo-1(+)* was injected at 7.5 ng/μl with a coinjection marker

of *Pmyo-2::gfp* at 5 ng/μl into *slo-1(js118)* using pBSKS as a carrier to create additional lines with the same genotype as BZ802, excepting the marker used for coinjection.

Plasmids: To create *Punc-17::slo-1(+)* and *Pcho-1::slo-1(+)*, promoters were excised from *unc-17::UNC-18::YFP* and *cho-1::UNC-18::YFP* respectively (Schindelman et al, 2006), blunt ended and ligated into pBK3.1 (*slo-1* cDNA controlled by *snb-1* promoter) after the *snb-1* promoter was removed.

b) *Punc-119::egl-19::yfp*, plasmid and transgenic strain.

To create this plasmid we followed the protocol used by (Garcia et al, 2001) to create *Pmyo-3::egl-19::gfp* with the following modifications. The promoter vector used was pBSKS containing the *unc-119* promoter sequence inserted directionally into the NotI and PstI sites (see below for *unc-119* promoter primers). A SalI site was added to the forward primer used to amplify *egl-19* to facilitate directional cloning to the *unc-119* promoter and a YFP version of pPD95.75 (pSX95.75) was used. The genomic *egl-19* PCR fragment was cut with SalI and three-way ligated to SalI/KpnI cut pBSKS containing the *unc-119* promoter and SmaI/XbaI cut pSX95.75 at a molar ratio of 8:2:1, PCR product to *unc-119* promoter vector to YFP vector. The *Punc-119::egl-19::yfp* ligation mix was injected at 200 ng/μl.

c) *Pcho-1:unc-18::yfp* plasmids and transgenic strains.

For *Pcho-1:unc-18::yfp* plasmid construction, see Schindelman et al (2006). This plasmid was injected at 75 ng/μl with a coinjection marker of *Pmyo-2::gfp* at 5 ng/μl into *unc-18(e81)* animals using pBSKS as a carrier to create *unc-18(e81); syEx788[Pcho-1:unc-18::yfp Pmyo::gfp]*.

d) Strains created for this work:

The following strains are from this work:

PS5407 (*egl-19(n582); syEx993[unc-119::egl-19::yfp]* line A),

PS5406 (*egl-19(n582); syEx992[unc-119::egl-19::yfp]* line B),

PS5410 (*slo-1(js118): syEx996[Pacr-2::slo-1]* line C),

PS5382 (*slo-1(js118): syEx988[Pacr-2::slo-1]* line D),

PS5387 (*slo-1(js118): syEx991[Pacr-2::slo-1]* line B),

PS5379 (*slo-1(js118): syEx985[Punc-25::slo-1]* line A),

PS5408 (*slo-1(js118): syEx985[Punc-25::slo-1]* line A),

PS5380 (*slo-1(js118): syEx986[Punc-17::slo-1]* line A),

PS5375 (*slo-1(js118): syEx982[Punc-17::slo-1]* line B),

PS5381 (*slo-1(js118): syEx987[Punc-17::slo-1]* line C),

PS5383 (*slo-1(js118): syEx989[Pcho-1::slo-1]* line A),

PS5384 (*slo-1(js118): syEx990[Pcho-1::slo-1]* line B),

PS5409 (*slo-1(js118): syEx995[Pcho-1::slo-1]* line C),

PS5191 (*unc-18(e81); syEx981[cho-1::unc-18::yfp]*).

References

Davies AG et al. (2003). A central role of the BK potassium channel in behavioral responses to ethanol in *C. elegans*. *Cell* 115: 655-666.

Schindelman G, Whittaker AJ, Thum JY, Gharib S, Sternberg PW (2006). Initiation of male sperm-transfer behavior in Caenorhabditis elegans requires input from the ventral nerve cord. *BMC Biol.* 4: 26.

Wang ZW, Saifee O, Nonet ML, Salkoff L (2001). SLO-1 potassium channels control quantal

content of neurotransmitter release at the C. elegans neuromuscular junction. *Neuron* 32: 867-81.