Dopaminergic neurons modulate locomotion in *Caenorhabditis elegans*

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Abstract

Adaptation in the sensory-mechanical loop during locomotion is a powerful mechanism that allows organisms to survive in different conditions and environments. For example, humans can walk on earth gravity, but they switch to hopping on, for example, moon gravity. The nematode *Caenorhabditis elegans* also shows adaptability by employing thrashing behaviour in low viscosity media and crawling in high viscosity media. The mechanism that enables this adaptability is yet unknown. It has been attributed previously to neuro-modulation by dopamine and serotonin.

The aim of this study is to physiologically investigate the neuronal mechanisms of modulation of locomotion by dopamine. It unravels a new role for the dopaminergic mechanosensory neurons, which is sensing the mechanical impact of the environment. The significance of such characterization is improving our understanding of dopamine gait switching which gets impaired in Parkinsons disease.

Keywords: *Caenorhabditis elegans*, Dopamine, Gait, Locomotion

1. Introduction

In nearly all the living organisms, locomotion is an important strategy for survival. It is important for feeding, avoiding predators, and finding optimal environmental conditions for survival. In order to maximize efficiency of locomotion in different environments and also in different situations (e.g., the presence of a predator), organisms need to have an adaptive strategy which needs a sensory system.

C. elegans modulates its locomotion pattern. This appears to be a modulation of undulation frequency, undulation wavelength, and velocity of locomotion. There has been a debate as to whether this modulation translates to two distinct locomotion patterns of swimming and crawling [Mesce & Pierce-Shimomura, 2010, Pierce-Shimomura et al., 2008, Vidal-Gadea et al., 2011]; or whether it is one locomotion pattern that is continuously modulated [Berri et al., 2009, Boyle et al., 2011, Fang-Yen et al., 2010, Korta et al., 2007].

Dopamine is a biogenic amine that has been shown to be associated with the process. In *C. elegans* hermaphrodite, there are 8 dopaminergic neurons distributed into 3 neuron types, namely CEP, ADE, and PDE. To date, five dopamine receptors have been identified in *C. elegans*. Two of them are homologous to mammalian D1-type receptors (*dop-1* and *dop-4*). The other three are homologues to D2-type receptors (*dop-2*, *dop-3*, and *dop-6*). These receptors are widely expressed in *C. elegans* nervous system.

Dopamine is responsible for a spectrum of behaviours. It has been shown that it is responsible for slowing of locomotion on the encounter of food [Sawin et al., 2000] and in local search behaviour along with serotonin [Hills et al., 2004]. Dopaminergic neurons are known to be mechanosensory [Bettinger

& McIntire, 2004, Duerr et al., 1999, Hills et al., 2004, Liu & Sternberg, 1995, Loer & Kenyon, 1993, Sanyal et al., 2004, Sawin et al., 2000] that have been shown to respond to harsh touch stimulation [Sanders et al., 2013]. A study has shown that dopamine is also responsible for gait switching from swimming to crawling [Vidal-Gadea et al., 2011]. They have also shown that serotonin is important and sufficient to switch from crawling to swimming.

In humans, gait dysfunction is a main symptom of the Parkinson's disease. It causes failure to modulate locomotion pattern by gait switching [Jankovic, 2008]. Other symptoms include trembling, stiffness, slowness of movement, and walking difficulty. It is caused by death of dopaminergic neurons in the substantia nigra. The cause of death of these cells is so far unclear [Obeso et al., 2010].

In this study, I measure the calcium activity of dopaminergic neurons as the worms switch between crawling and swimming due to crossing a viscosity separation to demonstrate that the worms respond to environment sensation by change in basal activity level and show the bidirectional nature of the dopamine effect. While the previous study [Vidal-Gadea et al., 2011] has shown that dopamine is responsible for switching from crawling to swimming as dopaminergic neurons are active at switching. Here, I show that dopaminergic neurons also respond to the environment as the worm is switching from crawling to swimming by a decrease in their basal activation which further supports the idea that dopaminergic neurons sense the pressure induced by the environment.

2. Methods

2.1. Behavioural assay

dat-1p::*GCaMP3* worms (Gift from Prof. Dr. David Biron, The University of Chicago) are placed between two glass slides with a paper separator of thickness 0.1-0.2 mm. The paper is coated with grease to secure the slides together (Figure 1). Worms are moved to a foodless plate before assay while the glass cassette is prepared. A tiny droplet of 65% dextran is placed on a glass slide so that the whole droplet is visible under microscope's field of view. The worm is picked and added to this droplet by a platinum wire worm pick. The second glass slide is then placed on top with the separator in the middle. The top plate is sheared by some distance from the lower plate to ease addition of the low viscosity medium later. Imaging starts while the worm is confined in the tiny droplet (Figure 1a). After the imaging starts, 30% dextran is added with a micropipette to fill the area around the 65% dextran droplet and then tracking starts (Figure 1b,c). Imaging continues until the whole worm's body passes through the viscosity separation to the lower viscosity region(Figure 1d). 30% dextran is used as opposed to pure buffer in order to ease the imaging as the worms swim in this medium but are slower compared to pure buffer. Ten worms were assayed for this experiment.

The point when the droplet fills in the gaps until half the droplet is chosen to be the zero time point and after that 70 seconds lengths of calcium imaging data are analysed. Controls are obtained through worms that stay confined within the small droplet as the area surrounding is not filled in order to compensate for photo-bleaching. Ten worms were assayed for the control experiment. Image sequences are imported to ImageJ software [Schindelin

et al., 2012] and the fluorescent signal is tracked manually using manual tracking tool [Cordelieres, 2005]. A 30 pixel region of interest around the tracking point is used to extract fluorescence signal. This is a bigger area than the cell size, but it is used to capture blurred signals due to quick movement during swimming. The signal is then smoothed to remove movement artefacts. A point not containing the worm is chosen as a background signal and the average of 100 points before zero is used for normalization (Figure 1).

2.2. Imaging

In this chapter. Ca^{+2} imaging was done under Nikon A1R high-speed confocal microscope illuminated by a mercury lamp Nikon Intensilight C-HGFIE with GFP filter. Acquisition is done by Andor Zyla 5.5 high resolution camera (Andor Technology Ltd., US) with Micromanager software [Edelstein et al., 2014]. Exposure time was set to 100 ms so the resulting frame rate is 10 FPS and 4 x 4 binning was done on each image so that the final resolution is 640 x 540 pixels. Autotracking was done using Hawkvision tracking system (Hawkvision Co. Ltd., Japan) with software developed by Saitama university in the whole body tracking mode to keep the worm in the field of view.

3. Results and Discussion

3.1. Dopaminergic neurons sense the physical environment

PDE neurons have shown deactivation response when the worms cross the viscosity separation (Figure 2a). Frequency of head undulation of *C.*

elegans increases accordingly, however even if it does not increase to reach the swimming typical frequency (Figure 2b), still the corresponding PDE response goes down. This suggests that PDE neurons respond by deactivation to the environmental pressure and not to the body bends. However, they still showed response to body bends (data not shown).

The mean calcium response to crossing the separation is shown in figure 2c,d where zero time point refers to the time when 30% dextran is visible to have surrounded half of the 65% dextran droplet (Figure 1). I also show the response up to 70 seconds after the zero time point since the worm's body, and hence the PDE neurons, takes 10-60 seconds to fully move from the 65% dextran and be fully swimming in 30% dextran which is apparent in the gradual increase in undulation frequency (Figure 2d). The transition time is variable among worms, so the mean calcium signal shows gradual change until it stabilizes at about 60 seconds to about 75% of its value in high viscosity. In some cases we observe a sudden drop while in other cases the drop is gradual (Figure 2a) depending on the speed of crossing the separation. The separation described is also designed to be a very steep gradient of viscosity but as time passes and due to diffusion and stirring effect caused by the worm movement, the gradient can get smoother and hence the transient in the physical forces sensed gets smoother. The signals from 60 to 70 seconds are significantly different (p=0.000) compared to signals from 10 seconds before addition of low viscosity medium (Figure 2e,f) .

3.2. Calcium signal of PDE and frequency of undulation correlate negatively

In order to find a relation between frequency of undulation and calcium signal, the unsmoothed calcium signals were plotted against frequency of

undulation. First, paired-sample t-test was performed on the data and the null hypothesis was rejected (p=0.000). Linear fitting was done by fitting a first degree polynomial $f(x) = px + c$ where p is the correlation coefficient. The fit showed a correlation coefficient (p = -0.2419) with a goodness of fit of r^2=0.1232 which is low due to fitting of all animals' data together (Figure 2e). The same fit was attempted on each time series which shows better results since signals are more self-consistent (Table 1). Another reason for the non-uniformity of distribution is the movement artefacts caused by the neuron sensing body contractions since the dataset used in the fit is unsmoothed. Some vertical clusters and diagonal lines are observed also in the dataset in figure 2g. These are caused by the cubic spline interpolation process that is done to fill in the gaps in fluorescence levels data and frequency caused by the lack of reliability of measurement mentioned in the methods section.

The study shows that mechanosensation has an effect on normal locomotion behaviour agreeing with a previous report [Li et al., 2006]. This is the first report to attribute such role to PDE neurons through direct measurement of activity. I show that viscosity differences can be measured by mechansensory neurons and it yields significant change in basal activation level of the neurons which can imply a change in dopamine release.

3.3. Hypothesized circuit for forward locomotion

Previous reports have shown that activation of dopaminergic neurons causes switching from swimming to crawling and activation of serotonergic neurons causes switching from crawling to swimming [Vidal-Gadea et al., 2011]. Here, it is shown that the latter has an associated decrease in dopamine level. Thus, it can be suggested that the balance between dopamine and sero-

Table 1: Linear fit results of fluorescence intensity difference vs. undulation frequency for each worm. In the weighted average, each value of p is weighted by the goodness of fit (r^2).

Worm	p	$\mathbf{r}^2$
1	-0.01745	0.174
2	-0.02725	0.4107
3	-0.01693	0.09379
4	-0.05399	0.4188
5	0.0125	0.008285
6	-0.0262	0.2099
7	-0.04159	0.1135
8	-0.02897	0.3299
9	-0.01399	0.0838
10	-0.02735	0.1083
Average	-0.0241	0.1957
Weighted Average	-0.0318	N/A
Overall	-0.2419	0.1232

tonin levels is responsible for mediating crawling-swimming switching. This can be further tested by simultaneous imaging of activation level of both dopaminergic and serotonergic neurons. Moreover, in a previous study, it was found that RID, RIS, and PQR are the neurons responsible for dopamine-mediated gait switching from swimming to crawling [Topper, 2013]. RID and RIS also express serotonin receptors *ser-2* and *ser-4* respectively so it can be suggested that they are the ones that compute the dopamine-serotonin balance and modulate locomotion accordingly. RIS neuron is connected to the head motor neurons and has been shown before to inhibit head movement during sleep-like state [Turek et al., 2013], so it might be a good candidate for regulating head undulation frequency. RID has gap junctions with the forward locomotion command interneurons AVB that activates body motor neurons. Calcium imaging of these two neurons would be a logical next step. Hypothesized circuit for locomotion modulation based on these observations is shown in figure 3.

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References

Berri, S., Boyle, J. H., Tassieri, M., Hope, I. A., & Cohen, N. (2009). Forward locomotion of the nematode *C. elegans* is achieved through modulation of a single gait. *HFSP Journal*, *3*(3), 186–193.

Bettinger, J., & McIntire, S. (2004). State-dependency in c. elegans. *Genes, Brain and Behavior*, *3*(5), 266–272.

Boyle, J. H., Berri, S., Tassieri, M., Hope, I. A., & Cohen, N. (2011). Gait modulation in *C. elegans*: It's not a choice, it's a reflex! *Frontiers in behavioral neuroscience*, *5*, 10.

Cordelieres, F. P. (2005). Manual tracking. *Institut Curie, Orsay (France)*.

Duerr, J. S., Frisby, D. L., Gaskin, J., Duke, A., Asermely, K., Huddleston, D., Eiden, L. E., & Rand, J. B. (1999). The cat-1 gene of caenorhabditis elegansencodes a vesicular monoamine transporter required for specific monoamine-dependent behaviors. *The Journal of neuroscience*, *19*(1), 72–84.

Edelstein, A. D., Tsuchida, M. A., Amodaj, N., Pinkard, H., Vale, R. D., & Stuurman, N. (2014). Advanced methods of microscope control using μmanager software. *Journal of biological methods*, *1*(2).

Fang-Yen, C., Wyart, M., Xie, J., Kawai, R., Kodger, T., Chen, S., Wen, Q., & Samuel, A. D. (2010). Biomechanical analysis of gait adaptation in the

nematode *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences*, *107*(47), 20323–20328.

Hills, T., Brockie, P. J., & Maricq, A. V. (2004). Dopamine and glutamate control area-restricted search behavior in *Caenorhabditis elegans*. *The Journal of neuroscience*, *24*(5), 1217–1225.

Jankovic, J. (2008). Parkinsons disease: clinical features and diagnosis. *Journal of Neurology, Neurosurgery & Psychiatry*, *79*(4), 368–376.

Korta, J., Clark, D. A., Gabel, C. V., Mahadevan, L., & Samuel, A. D. (2007). Mechanosensation and mechanical load modulate the locomotory gait of swimming *C. elegans*. *Journal of Experimental Biology*, *210*(13), 2383–2389.

Li, W., Feng, Z., Sternberg, P. W., & Xu, X. S. (2006). A *C. elegans* stretch receptor neuron revealed by a mechanosensitive trp channel homologue. *Nature*, *440*(7084), 684–687.

Liu, K. S., & Sternberg, P. W. (1995). Sensory regulation of male mating behavior in caenorhabditis elegans. *Neuron*, *14*(1), 79–89.

Loer, C. M., & Kenyon, C. J. (1993). Serotonin-deficient mutants and male mating behavior in the nematode caenorhabditis elegans. *The Journal of neuroscience*, *13*(12), 5407–5417.

Mesce, K. A., & Pierce-Shimomura, J. T. (2010). Shared strategies for behavioral switching: understanding how locomotor patterns are turned on and off. *Frontiers in behavioral neuroscience*, *4*.

Obeso, J. A., Rodriguez-Oroz, M. C., Goetz, C. G., Marin, C., Kordower, J. H., Rodriguez, M., Hirsch, E. C., Farrer, M., Schapira, A. H., & Halliday, G. (2010). Missing pieces in the parkinson's disease puzzle. *Nature medicine*, *16*(6), 653–661.

Pierce-Shimomura, J. T., Chen, B. L., Mun, J. J., Ho, R., Sarkis, R., & McIntire, S. L. (2008). Genetic analysis of crawling and swimming locomotory patterns in *C. elegans*. *Proceedings of the National Academy of Sciences*, *105*(52), 20982–20987.

Sanders, J., Nagy, S., Fetterman, G., Wright, C., Treinin, M., & Biron, D. (2013). The *Caenorhabditis elegans* interneuron ala is (also) a high-threshold mechanosensor. *BMC neuroscience*, *14*(1), 156.

Sanyal, S., Festa, F., Sakano, S., Zhang, Z., Steineck, G., Norming, U., Wijkström, H., Larsson, P., Kumar, R., & Hemminki, K. (2004). Polymorphisms in dna repair and metabolic genes in bladder cancer. *Carcinogenesis*, *25*(5), 729–734.

Sawin, E. R., Ranganathan, R., & Horvitz, H. R. (2000). *C. elegans* locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. *Neuron*, *26*(3), 619–631.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., & Schmid, B. (2012). Fiji: an open-source platform for biological-image analysis. *Nature methods*, *9*(7), 676–682.

Topper, S. M. (2013). *Gait transitions in C. elegans*. Ph.D. thesis, University of Texas at Austin.

Turek, M., Lewandrowski, I., & Bringmann, H. (2013). An ap2 transcription factor is required for a sleep-active neuron to induce sleep-like quiescence in *C. elegans*. *Current Biology*, *23*(22), 2215–2223.

Vidal-Gadea, A., Topper, S., Young, L., Crisp, A., Kressin, L., Elbel, E., Maples, T., Brauner, M., Erbguth, K., & Axelrod, A. (2011). *Caenorhabditis elegans* selects distinct crawling and swimming gaits via dopamine and serotonin. *Proceedings of the National Academy of Sciences*, *108*(42), 17504–17509.

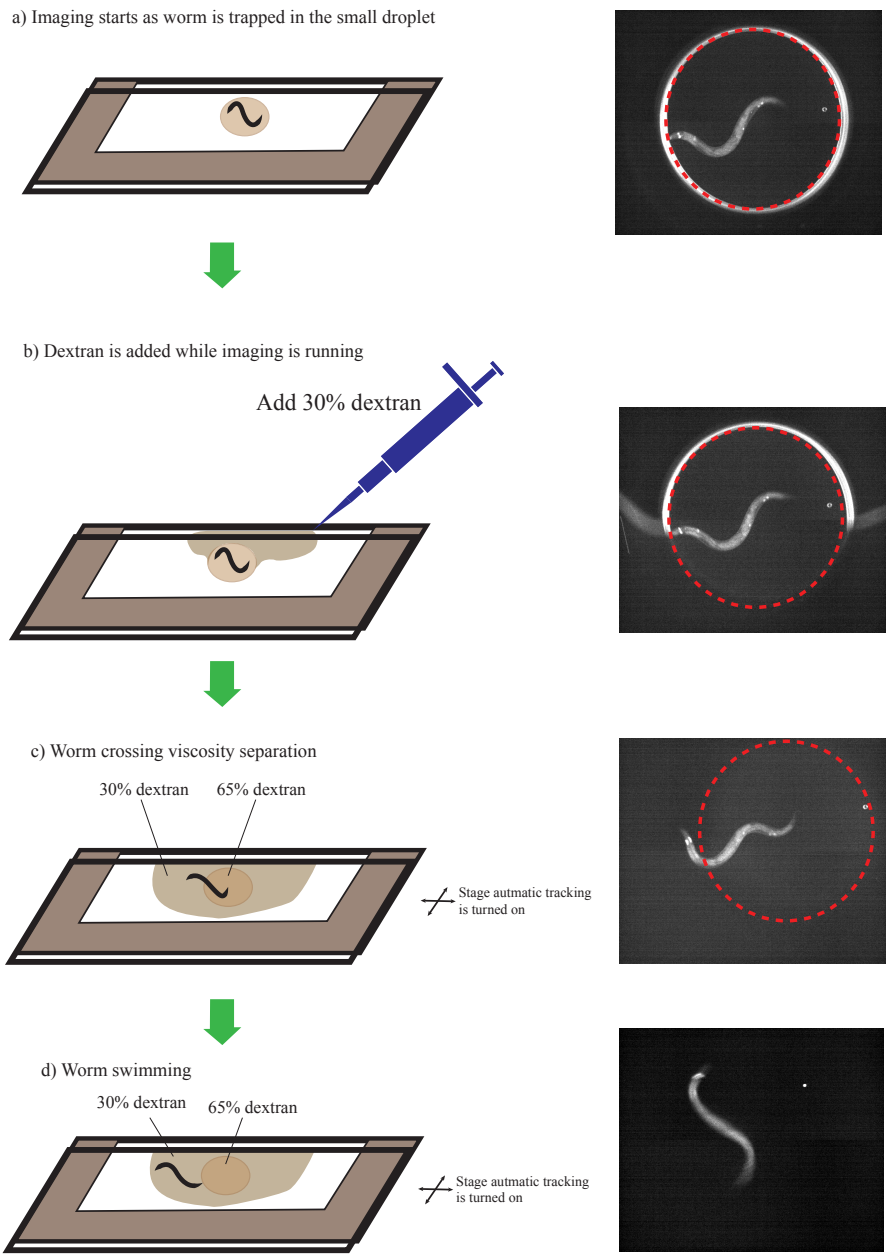

Figure 1: A description of the steps of the viscosity separation where the worm is initially confined in a small droplet of 65% dextran (a) and then dextran 30% is added with micropipette (b). The worm then starts to move out of the small droplet (c) until the whole body gets to the lower viscosity and swimming is maintained.

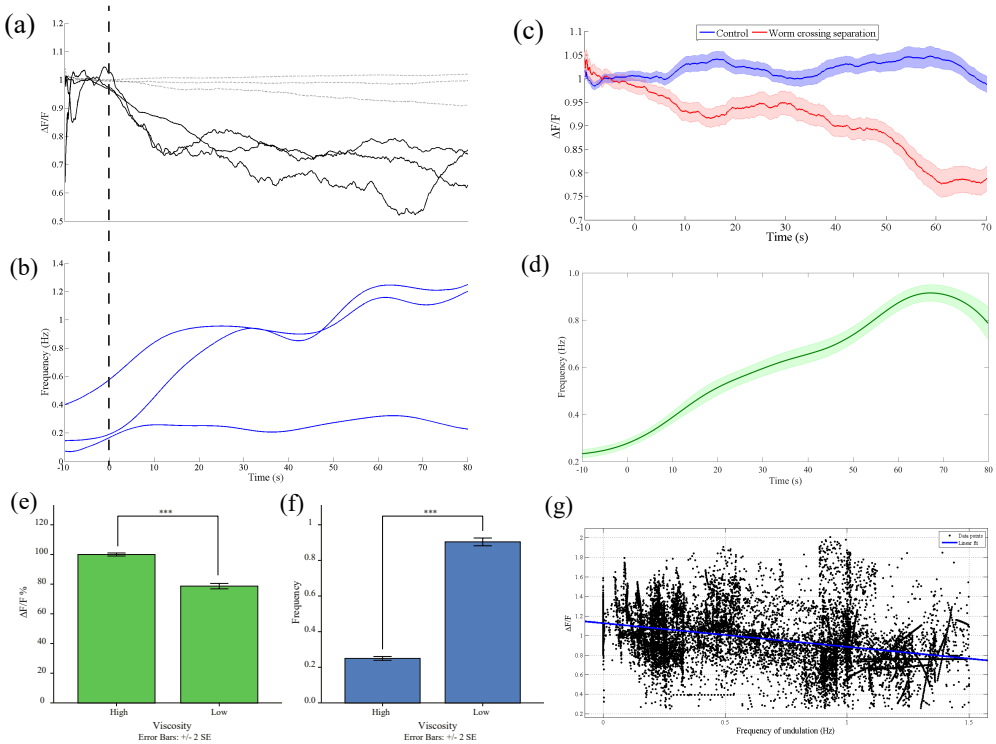

Figure 2: Fluorescence signal of PDE neurons and corresponding behavioural response to the worm crossing viscosity separation: (a) PDE neurons' activity and (b) frequency of undulation response to crossing viscosity separation for three worms. Solid lines represent the worms that cross the separation while dotted line represents three control worms which remain at high viscosity. Frequency responses are interpolated and smoothed for each animal. (c) Average PDE response to crossing the separation where the zero point is the point of addition of the droplet of liquid (n=10). The control represents worms that remain at high viscosity. (d) Average frequency response to crossing the separation (n=10). The frequency was measured each 10 seconds and then linear interpolation was done to get the smooth curve. Error bars correspond to ±2 SE. (e & f) Mean of the first 10 seconds and last 10 seconds of data from (c) and (d) where the worm in each case is in a completely homogeneous viscosity in both cases the data leads to a significant difference (p=0.000). (g) Mathematically fitting the data from the fluorescence intensity difference in PDE neurons with the frequency of undulation. This figure tries to fit all the data of different worms together.

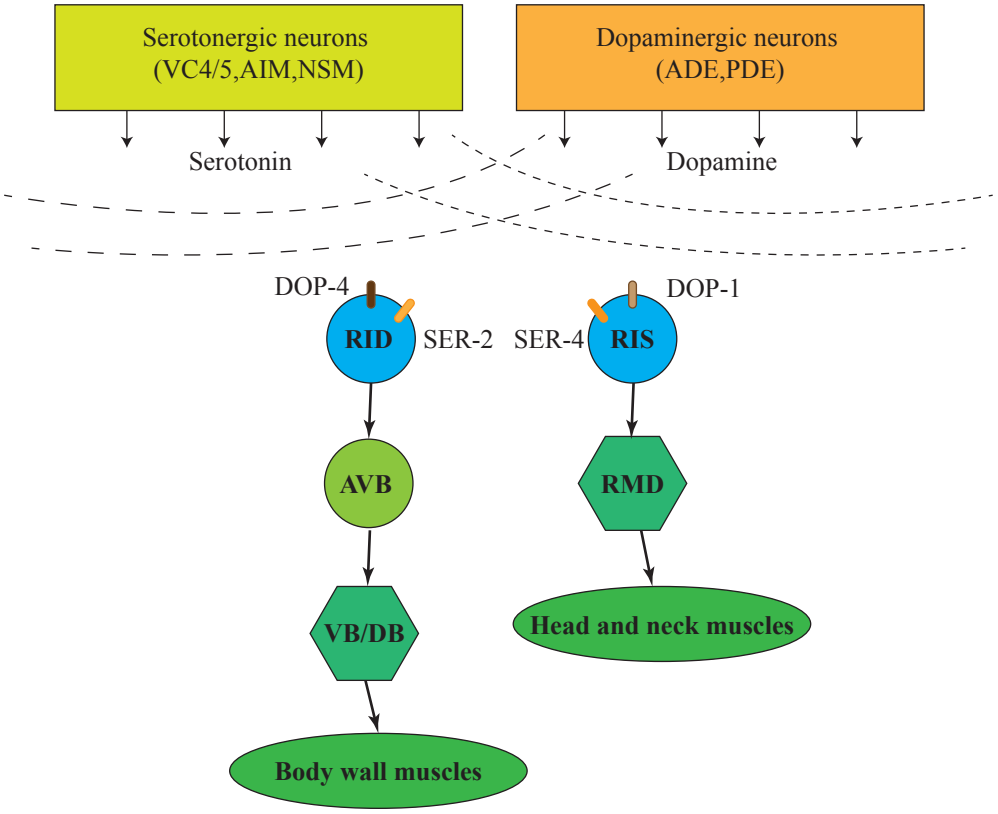

Figure 3: Hypothesized circuit for forward locomotion modulation in *C. elegans* based on monoaminergic regulation of locomotory activity.