The HSNs regulate the defecation motor program in *Caenorhabditis elegans*

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Figure 1.

(A) Wiring diagrams of the reproductive circuit (top, yellow) and defecation motor circuit (bottom, blue). HSN (green) and VC (blue) neurons synapse onto each other and the vm2 muscles for egg laying. Data from White J.G. et al. (1986) indicate HSN and VC also make and receive synapses from AVL and DVB, excitatory GABA motor neurons that regulate the contraction of the enteric muscles (em) for defecation. Arrows indicate chemical synapses, and + or – indicates a presumptive excitatory or inhibitory synapse, respectively. Bar-headed lines indicate gap junctions (e.g. electrical synapses).

(B) Representative HSN Ca²⁺ traces during the L4.7-8 larval stage (top) and adults (bottom). Vertical lines indicate the expulsion step of the defecation motor program (DMP); arrowheads indicate adult egg-laying events.
(C) Cumulative distribution plots showing instantaneous frequency of the DMP events (min\(^{-1}\)) with no observed HSN Ca\(^{2+}\) transient (black) and those with one or more HSN Ca\(^{2+}\) transients (green) in L4.7-8 (closed circles) and adult animals (open circles). Pound indicates p=0.0058; asterisk indicates p<0.0001 (Kruskal-Wallis test with Dunn’s correction for multiple comparisons). Total DMP intervals used for analysis: L4.7-8 DMPs without an HSN Ca\(^{2+}\) transient (n=33); L4.7-8 DMPs with at least one HSN Ca\(^{2+}\) transient (n=62) from 9 animals; Adult DMPs without an HSN Ca\(^{2+}\) transient (n=39); Adult DMPs with at least one HSN Ca\(^{2+}\) transient (n=72) from 11 animals.

(D) Scatterplots showing the consequences of HSN optogenetic activation on DMP frequency. L4.7-8 and adult animals expressing Channelrhodopsin-2 (ChR2) in HSN neurons from the wzIs30 transgene were grown in the absence (–, grey) or presence (+, blue) of all-trans retinal (ATR), illuminated with continuous blue light for two minutes, and the timing of DMP events was recorded. The elapsed time between Expulsion events was used to calculate an instantaneous DMP frequency from each recorded interval (min\(^{-1}\)) from 10 animals. Error bars show 95% confidence intervals for the mean; * indicates p<0.0001; n.s. indicates p=0.2102 (L4.7-8) or p>0.999 (Adult) (One-way ANOVA with Bonferroni correction for multiple comparisons). Total DMP intervals used for analysis from 10 animals: L4.7-8, no ATR (n=43); L4.7-8, plus ATR (n=40); Adult, no ATR (n=67); Adult, plus ATR (n=55).

(E) Scatterplots showing average DMP frequencies (min\(^{-1}\)) from ten wild-type (grey), HSN-deficient egl-1(n487dm) and egl-1(n986dm) mutants (red), gain-of-function egl-47(n1082dm) (pink), and egl-8(sa47) PLC\(\beta\) null mutant adults (brown) after recording for 5 min. Error bars indicate the 95% confidence interval for the mean. Asterisk indicates p<0.0001; n.s. indicates p=0.5208 (One-way ANOVA with Bonferroni’s correction for multiple comparisons).

**Description**

We have recently described an unusual rhythmic Ca\(^{2+}\) activity in the developing Hermaphrodite Specific Neurons. We exploited the stereotyped morphology of the developing primary and secondary vulval epithelial cells in the fourth (final) larval stage to define discrete half-hour stages of development until the L4-adult molt as described (Ravi et al. 2018b). We observed a ~50 s rhythm of HSN activity in L4.9 animals which resembled the rhythm of the defecation motor program (DMP), prompting us to investigate whether there is a relationship between neural circuits that regulate reproduction and defecation behaviors in *C. elegans*. As shown in Figure 1A, the egg-laying HSN command neurons and VC motor neurons make and receive synapses from the excitatory GABAergic AVL and DVB motoneurons that regulate
defecation (White, J.G. et al. 1986). Serotonin and Gαo signaling, which regulate egg laying behavior, can also inhibit the defecation motor program (Ségalat et al. 1995; Waggoner et al. 1998; Hardaker et al. 2001; Tanis et al. 2008; Brewer et al. 2019). However, the functional relationship between what are thought to be independent motor circuits has not been examined. Because evidence shows that both the egg-laying active state and the DMP are linked to changes in forward and reverse locomotion (Hardaker et al. 2001; Nagy et al. 2015), we reasoned there may be a relationship between circuits that drive these two expulsive behaviors.

Using a transgene that co-expresses GCaMP5 and mCherry in the HSNs from the nlp-3 promoter (Collins et al. 2016), we performed ratiometric Ca\(^{2+}\) imaging in L4.7-8 juveniles and egg-laying adults and compared the timing of HSN Ca\(^{2+}\) transients and defecation events (Ravi et al. 2018a). We found that defecation intervals in L4.7-8 and adult animals were significantly longer when they were accompanied by one or more HSN Ca\(^{2+}\) transients (Fig. 1B and 1C). This suggested the HSNs might signal to inhibit the defecation motor rhythm. To test this, we used a transgene that expressed Channelrhodopsin-2 in the HSNs from the egl-6 promoter (Emtage et al. 2012) and tested whether acute optogenetic activation of the HSNs in L4.7-8 juveniles or adults affected the DMP rhythm. Blue light illumination of animals grown on ATR, an essential cofactor for ChR2, caused a mild reduction in DMP frequency in L4.7-8 animals, but this effect was not statistically significant (p=0.2102) and was not observed in adults (Fig. 1D). Interestingly, we observed that DMP frequency was significantly longer in 24-hour adult animals compared to L4.7-8 juveniles. Previous work has shown a significant decline in the DMP frequency in aging animals, although this is reduction was not apparently related to changes in feeding as measured by pharyngeal pumping (Croll et al. 1977; Bolanowski et al. 1981).
We next examined DMP frequency in animals with altered HSN neurotransmitter signaling. Because we observed that periods of elevated HSN Ca\(^{2+}\) activity had reduced DMP frequency (Fig. 1C), we hypothesized that mutations that reduce HSN neurotransmitter signaling would increase DMP frequency. Surprisingly, animals bearing two independent egl-1(dm) mutants that cause the HSNs to undergo premature cell death showed a significant decrease in DMP frequency (Fig. 1E). This indicates the HSNs are required for a normal DMP rhythm in adult animals. However, this defecation phenotype was not observed in egl-47(dm) mutant animals which have strong defects in HSN neurotransmitter release and similar defects in egg-laying behavior as egl-1(dm) mutants (Moresco and Koelle 2004; Tanis et al. 2009). egl-47(dm) animals still show occasional HSN Ca\(^{2+}\) transients (Ravi, unpublished observations), so the reduced DMP frequency in egl-1(dm) animals may indicate the HSNs are developmentally required for a normal DMP rhythm or that even low levels of serotonin or NLP-3 neuropeptide release from HSN are sufficient to impart a normal DMP rhythm(Brewer et al. 2019). We propose that animals actively suppress defecation during the egg-laying active state, possibly to direct circuit- and behavior-specific changes in body wall muscle contractility that coordinate increases in hydrostatic pressure that drive expulsion of gut or uterine contents. Consistent with this model, a common set of signaling molecules regulate the activity of circuits that modulate egg-laying and defecation behaviors (Reiner et al. 1995). Our identification of a functional relationship for HSN in regulating DMP frequency may provide an avenue to understand how both physical circuits and extrasynaptic signaling drive alternate behavior states (Bentley et al. 2016).

**Reagents**
Strains available from CGC: LX2004 vsIs183 [nlp-3p::GCaMP5::nlp-3 3'UTR + nlp-3p::mCherry::nlp-3 3'UTR + lin-15(+)] lite-1(ce314) lin-15(n765ts) X; LX1836 wziIs30 [egl-6::ChR2-YFP::unc-54 3'UTR + lin-15(+)] IV, lite-1(ce314) lin-15(n765ts) X; MT1082 egl-1(n487dm) V; MT2248 egl-47(n1081dm) V; JT47 egl-8(sa47) V. Strain available upon request: MIA26 egl-1(n986dm) V. All-trans retinal (100 mM stock in ethanol) was from Sigma and added to pre-warmed OP50 bacterial cultures in B Broth as described (Collins et al. 2016; Ravi et al. 2018b). DMP frequency was measured based on the timing of the final expulsion step (Liu and Thomas 1994). Ratiometric Ca^{2+} imaging was performed in freely behaving animals as previously described (Collins and Koelle 2013; Collins et al. 2016; Ravi et al. 2018a; b; Brewer et al. 2019).

References


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Kevin Collins: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project Administration, Software, Supervision, Visualization, Writing

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