Functions and mechanisms of a Central Pattern Generator circuit in *C. elegans*

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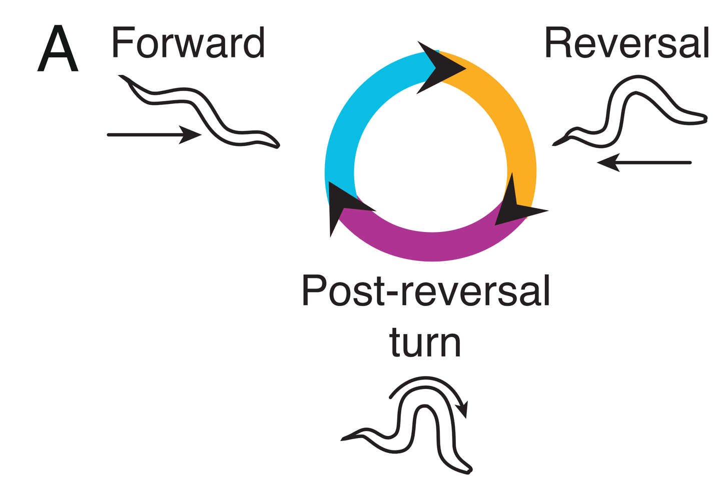
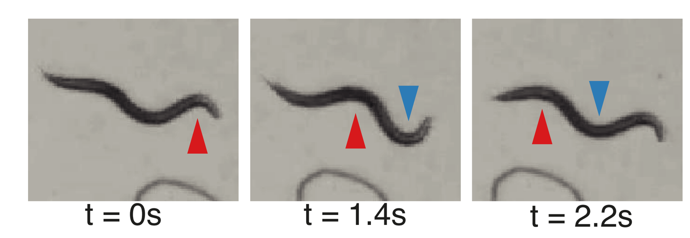
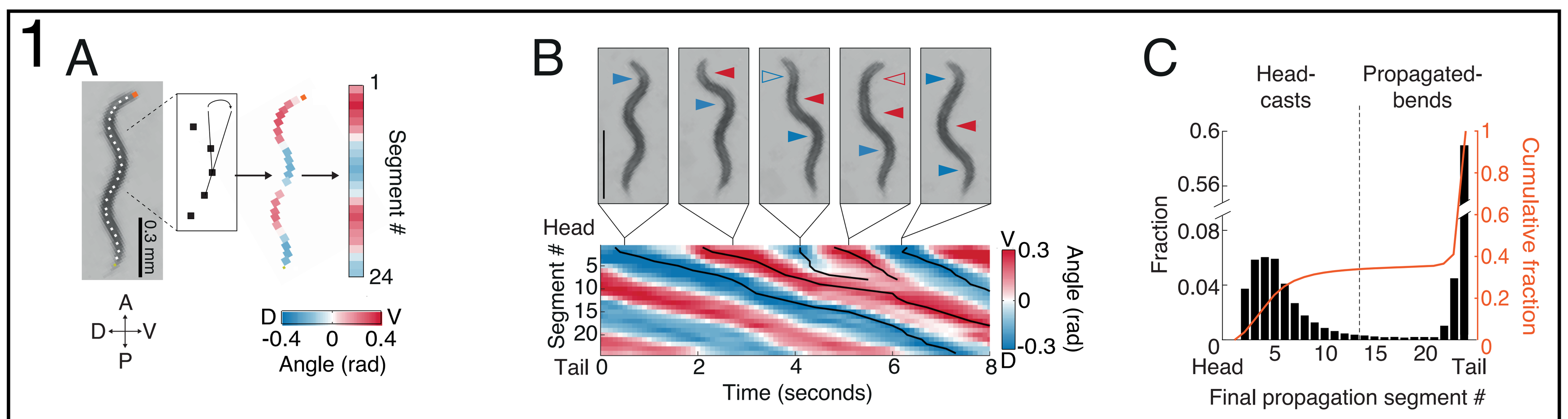
Introduction

Rhythmic movements are crucial for animal survival, breathing, walking and swimming are prime examples of this. The underlying rhythmic activity is produced by circuits of central pattern generating (CPG) neurons that can operate independently of patterned sensory information. Other neuronal ensembles in the brain also show organized rhythmic activity; these oscillations may play a crucial role in neuronal computations. I am exploring the mechanisms and functions of neuronal oscillatory activity in C. elegans, an organism that can be interrogated at the systems, single cell and molecular level.

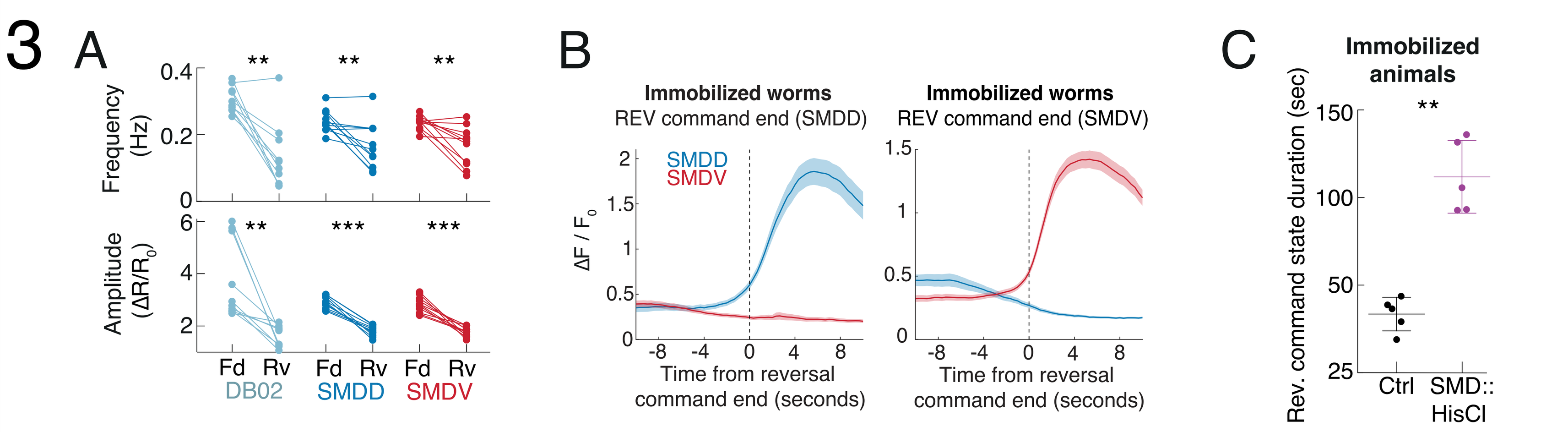
C. elegans food search behavior can be simplified as a cycle of forward- and reverse-directed locomotion, the latter being followed by post-reversal reorientation turns (Panel A). We previously described neuronal dynamics underlying the switches between forward locomotion and reversals (Ref. 1). The forward locomotion state consists of rhythmic bends along the body, propagating from head to tail (Panel B). We started by identifying shorter timescale, rhythmic behaviors within the predominant forward locomotion state.



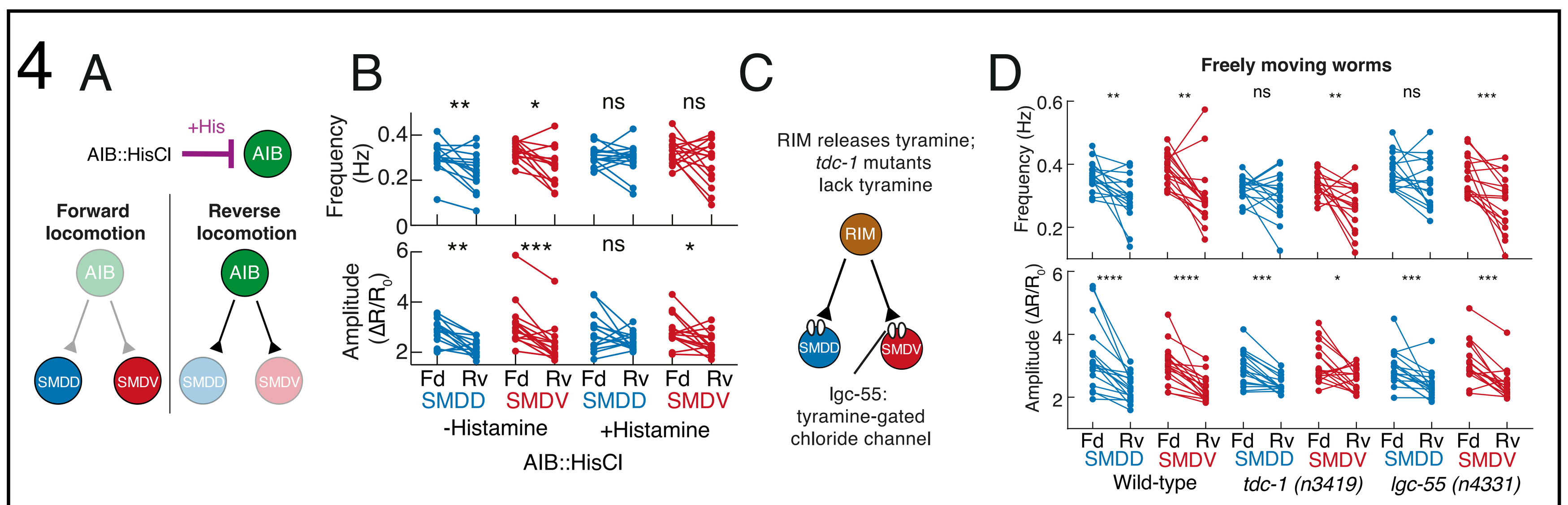




Detailed behavioral analysis reveals two types of head bends: slower "propagated bends" and faster "head casts".
(A-B) Posture measurement from videos **(A)**; timeseries as a kymogram **(B)**. Black lines trace head-bends propagation. **(C)** Head-bend propagations are bimodal, indicating two distinct types.



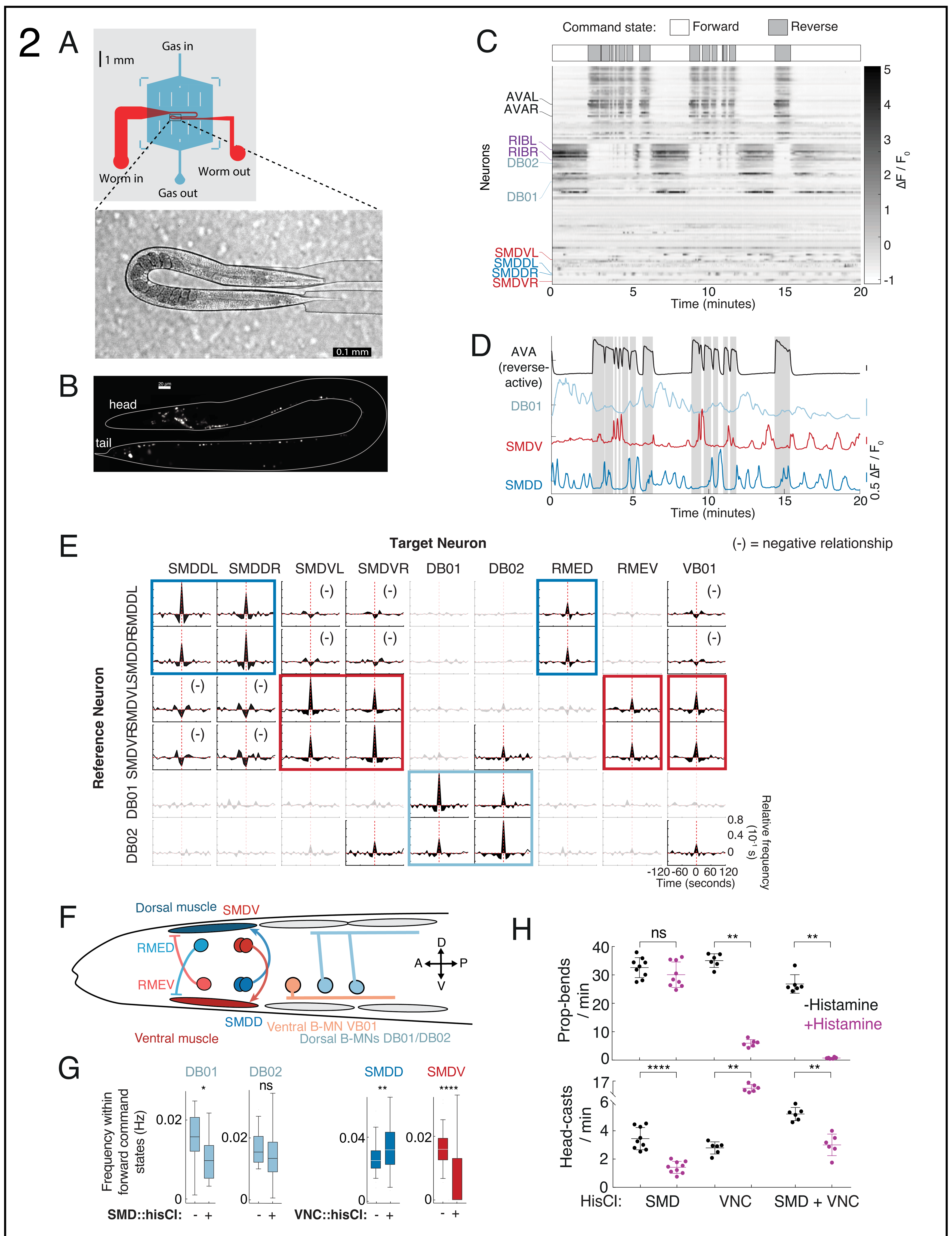
Neuronal oscillators are multi-functional depending on behavioral state.
(A) Forward/reverse state modulates DB02 and SMD activity in freely-moving animals. **(B)** SMD activity is strongly coupled to reversal command end. **(C)** SMDs promote reverse-to-forward state transitions



Inputs from AIB and RIM interneurons modulate SMD oscillators.
(A, C) SMD activity could be modulated in forward/reverse through circuit interactions. Thus, we assessed the role of presynaptic AIB through hisCl inhibition **(A)** and RIM **(C)**. **(B)** AIB inhibition abolished the forward/reverse modulation of SMD frequency and SMDD amplitude. **(C)** RIM affects head oscillations via tyramine and SMDs express LGC-55. Thus, we tested tyramine biosynthesis (tdc-1) and lgc-55 mutants. **(D)** Both mutants were defective in the forward/reverse modulation of SMDD frequency, but no other parameters. For more information see Ref. 2.

References

1. Kato, S. et al. Global Brain Dynamics Embed the Motor Command Sequence of Caenorhabditis elegans. Cell 163, 1-50, doi:10.1016/j.cell.2015.09.034 (2015).
2. Kaplan, H. S., Salazar Thula, O. et al. Nested Neuronal Dynamics Orchestrate a Behavioral Hierarchy across Timescales. Neuron 105, 1-15, doi:10.1016/j.neuron.2019.10.037 (2020)



Ca^{2+} imaging screen identifies CPG candidate circuits for each behavior.
(A) Worms are paralyzed and immobilized in a microfluidic device for pan-neuronal imaging using a genetically-encoded fluorescent calcium indicator (GCaMP). **(B)** Maximum intensity projection of a whole-animal widefield deconvolution GCaMP6f recording. **(C)** Example recording. Each row shows the activity of one neuron. Spontaneous neuronal population dynamics correspond to behavioral command states (Ref. 1). **(D)** Neuronal activity traces of candidate motor neurons (MNs). **(E-F)** MN oscillators show significant covariograms with a limited set of other neurons, anatomical locations seen in **(F)**. **(G)** Inhibition of candidates using a histamine-gated chloride channel (hisCl) shows they oscillate independently. B-MNs targeted with a ventral nerve cord (VNC) driver. **(H)** SMD inhibition decreases head-cast frequency; VNC inhibition abolishes prop-bends.

Open questions

- We identified that motor neurons RME and VB01 show activity correlations to the SMDs and could therefore be part of CPG subcircuits (Fig. 2E). What role do these neurons play in rhythm generation and/or SMD activity?
- Do electrical connections play a role in SMD rhythmic activity? How important are gap junction inputs from circuit participants?
- SMDD and SMDV show antagonistic, rhythmic activity. How is this antagonism achieved and is it required for rhythm generation? Can SMDD and SMDV oscillate on their own? Are cholinergic and/or GABAergic signaling involved in establishing this antagonism?
- How are the propagated-bend (motor neurons DB01/DB02) and head-cast (SMD/RME/VB01) oscillators coupled to ensure fluent movement?