Microfluidic electrophysiological recordings from host-stage parasitic larvae: a tool for phenotyping neuromuscular activity & feeding behavior

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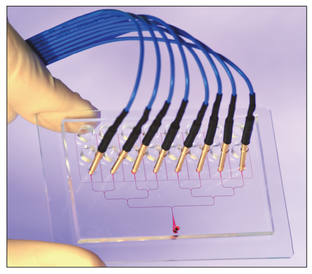
INTRODUCTION

Phenomics—the analysis of physiological changes caused by genetic or other manipulations—is an important component of molecular helminthology. Feeding behavior, produced by rhythmic electrical activity of neurons and muscles of the pharynx, is required for survival and provides a target for helminth control. Pharyngeal pumping provides a sensitive readout of a worm's general health; e.g., pharyngeal function declines during aging, expression of disease genes, etc. (**1**). We previously developed a microfluidic platform for *C. elegans* that provides medium-throughput, high-content quantification of muscular and neural activity during feeding (**2**). Because of the significant disease burden caused by nematode infections, our present goal was to adapt this platform for soil-transmitted helminths: specifically, host-stage larvae of *Ancylostoma ceylanicum* and *Ascaris suum* (**3**). NemaMetrix (**4**), a university spin-off company serving the worm community, offers a turn-key ScreenChip system that makes electro-physiological recording and analysis accessible to any nematode laboratory, in the context of phenomics, drug discovery or other applications.

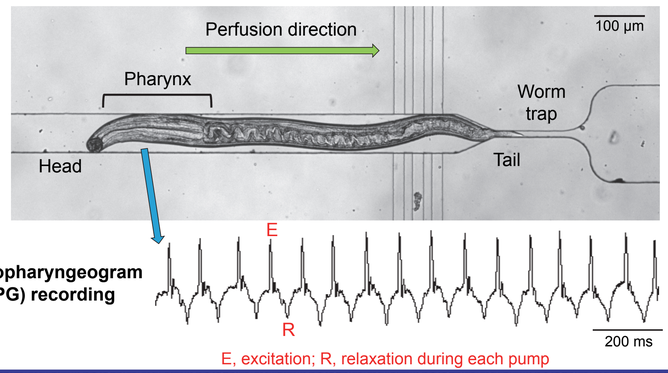
METHODS

Microfluidic 'chips' were fabricated by soft lithography (PDMS on glass) with channel diameters customized for *A. ceylanicum* L4 from hamsters and *A. suum* L3 from swine.



Electropharyngeograms (EPGs) produced by rhythmic contraction (pumping) of the pharynx were recorded from 8 worms simultaneously, at 34-38 °C in RPMI medium with additives. Experimental & test solutions were perfused over the worms while recording.

A. ceylanicum L4 in the recording module of a chip

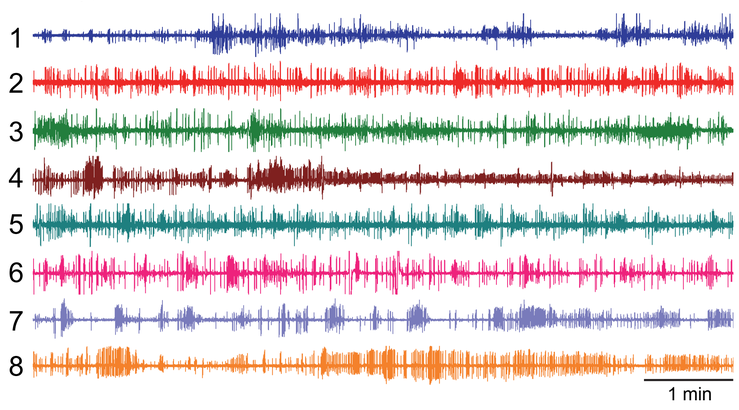


E, excitation; R, relaxation during each pump

RESULTS

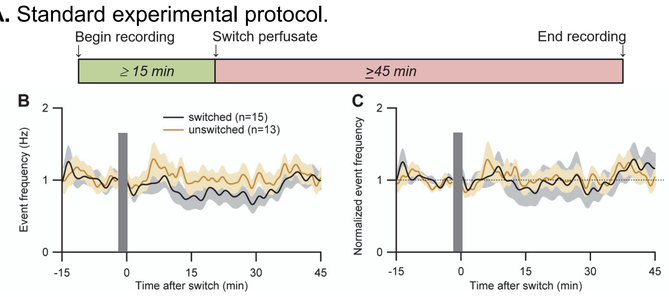
1. *A. ceylanicum* L4 produce robust, sustained activity in microfluidic chips

Traces show simultaneous EPG recordings from 8 worms (labeled 1 – 8) in one chip, in RPMI + serum.



2. Pump frequency is stable over time

A. Standard experimental protocol.

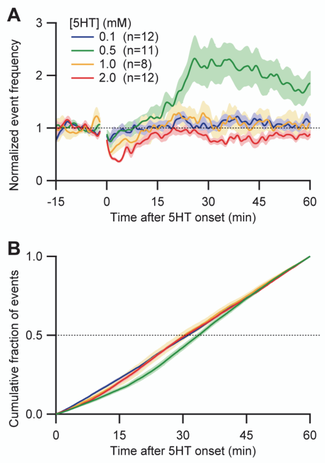


B. Pump frequency over time (mean ± SEM), with or without a perfusion switch (grey bar). Mean frequency was ~1 Hz (events/s). *n* (# of worms) in parentheses. *A. ceylanicum* L4 in RPMI + serum.

C. Data in **B** were normalized to event frequency from -12 to -2 min before switch. Frequency in the switched vs. unswitched groups did not differ ($P > 0.3$; 2-tailed Wilcoxon Mann-Whitney U test).

Thus, EPG activity in hookworm was stable for >1 h and unaffected by the mechanical stimulus of switching the perfusion stream.

3. 5HT effect depends on concentration



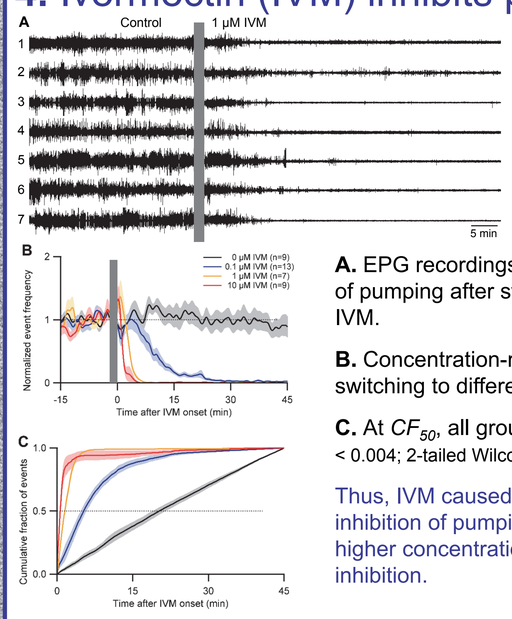
The neuromodulator serotonin (5HT) induces pharyngeal pumping in many nematodes (**5**). We tested its effects on *A. ceylanicum* L4 in RPMI + serum.

A. Concentration-response curves, switching perfusate to different [5HT]s.

B. Cumulative fraction (CF) of events after switch. At CF_{50} (dotted line), all groups were similar, except 0.5 mM 5HT, which increased pump frequency ($P < 0.04$; 2-tailed Wilcoxon Mann-Whitney U test).

Since robust, sustained pumping was obtained in hookworm even without 5HT, it was omitted from subsequent experiments.

4. Ivermectin (IVM) inhibits pumping



We tested IVM as a representative anthelmintic known to rapidly inhibit pharyngeal pumping (**2**). *A. ceylanicum* L4 in RPMI + serum.

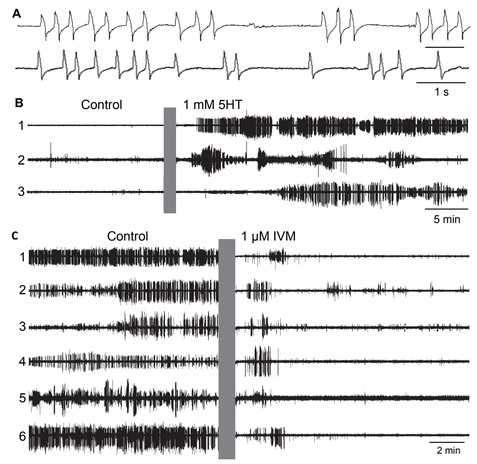
A. EPG recordings showing rapid inhibition of pumping after switching perfusate to 1 µM IVM.

B. Concentration-response curves after switching to different [IVM]s.

C. At CF_{50}, all groups differed significantly ($P < 0.004$; 2-tailed Wilcoxon Mann-Whitney U test).

Thus, IVM caused concentration-dependent inhibition of pumping in hookworm, with higher concentrations causing more rapid inhibition.

5. EPG recordings in *Ascaris suum* L3



We performed a less extensive characterization of *A. suum* L3.

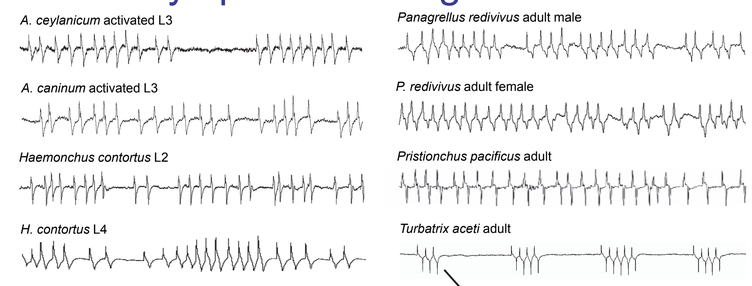
A. Representative EPG waveforms from 2 worms. RPMI + serum, 1 mM 5HT.

B. Pumping is induced by switching perfusate to 1 mM 5HT (in RPMI + serum) (**6**).

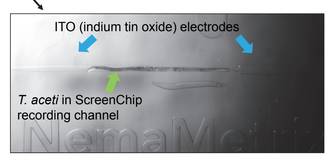
C. Pumping is inhibited by switching to 1 µM IVM (in RPMI, serum & 1 mM 5HT) (**6**).

Thus, host-stage *A. suum larvae* are suitable for use in microfluidic EPG chips.

6. Microfluidic EPG chips are compatible with many species & stages



In addition to *C. elegans* (L1 to adult), *A. ceylanicum* & *A. suum*, we made EPG recordings from other parasitic & free-living nematodes (**7**). NemaMetrix works with investigators to validate new species/stages in the platform.



SUMMARY

- Microfluidic EPG recordings provide a powerful new method for detecting and quantifying electrophysiological phenotypes in nematodes.
- The platform is compatible with many species, including host-stage larvae of soil-transmitted helminths *A. ceylanicum* and *A. suum*.
- Applications include phenotyping transgenic or mutant worms, compound library screening and analysis of nematode feeding behavior.
- Turn-key instrumentation & data acquisition/analysis software are available from NemaMetrix.

NemaMetrix
www.nemametrix.com

ACKNOWLEDGEMENTS

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(**4**) www.nemametrix.com
(**5**) e.g., Tahseen Q et al. 2003. Electropharyngeograms of pharyngeal pumping activity in six species of free-living nematodes. Nematology 6:49–54.
(**6**) Brownlee DJ et al. (1995) The action of serotonin and the nematode neuropeptide KSAYMRFamide on the pharyngeal muscle of the parasitic nematode, *Ascaris suum*. Parasitol 111:379–84; Brownlee DJ et al. (1997) Actions of the anthelmintic ivermectin on the pharyngeal muscle of the parasitic nematode, *Ascaris suum*. Parasitol 115:553–61.
(**7**) http://nemametrix.com/microfluidic-epg-recordings-diverse-nematode-species-2/ & unpub. data.