



NemaMetrix

E. coli-INDUCED PHARYNGEAL PUMPING IN *C. elegans*

USING THE SCREENCHIP SYSTEM

PURPOSE

Pharyngeal pumping behavior in *C. elegans* is employed to ingest bacteria, the worms' normal food. Under laboratory conditions, *C. elegans* are reared on agar plates seeded with the *E. coli* strain OP50, which stimulates feeding behavior (1, 2). Alternatively, pharyngeal pumping can be elicited in the absence of bacteria by exposing worms to serotonin (5-hydroxytryptamine; 5HT), an endogenous neuromodulator of feeding behavior (e.g., 3). To make electropharyngeogram (EPG) recordings in microfluidic chips, 5HT treatment can be used to elicit robust, sustained pharyngeal pumping (4; and see other NemaMetrix Technical Notes). In some circumstances, however, a more naturalistic feeding stimulus may be desired; e.g., when testing worms with mutations in the 5HT signaling system. Accordingly, we tested the ability of OP50 treatment to elicit pharyngeal pumping, using the ScreenChip system.

METHODS

C. elegans synchronization and cultivation

Synchronized N2 *C. elegans* were obtained by bleach synchronization. The resulting L1s were cultivated on plates containing standard nematode growth medium (NGM) seeded with *E. coli* OP50 and allowed to grow at 20 °C until they reached the first day of adulthood (2, 5).

Solutions

The 10 mM 5HT solution was made in a 15 mL conical vial by adding 10 mL of M9 buffer to 43 mg 5HT, and used within 2 h. OP50 was cultivated by seeding 200 ml of LB with OP50 and incubating the culture on a rocker for 2 d at 22 °C (2). The culture was then refrigerated to halt bacterial growth and stored for up to 1 month. For EPG experiments, cultured OP50 was spun at 5,000 RPM for 8 min in 50 mL conical tubes. The supernatant was removed, replaced with sterile H₂O and spun 2 more times. After the final spin, the supernatant was removed and the OP50 pellet dried at 22°C. The dried OP50 was weighed, suspended in M9 at 100 mg/ml (6) and determined to have an OD₆₀₀ (optical density at 600 nm) of ~3. The solution was refrigerated for up to 1 week.

Recording electropharyngeograms (EPGs)

A glass pipette with M9 buffer was used to rinse day 1 adults from a plate. They were transferred to a 1.5 mL Eppendorf tube (Epitube) and centrifuged for 2 min at 6,000 RPM. The supernatant was removed, replaced with fresh M9 and the worms spun down again. Experimental groups were prepared as follows: (i) M9 control group; the supernatant was removed, replaced with M9, and EPG recordings begun; (ii) 5HT control group; the supernatant was removed and replaced with M9 containing 10 mM 5HT. Worms were incubated for 20 min to activate pumping before starting EPG recordings; (iii) OP50 group; after the supernatant was removed and replaced with M9, worms were fasted in the Epitube for 2 h. They were then spun down at 6,000 RPM for 2 min, the supernatant was replaced



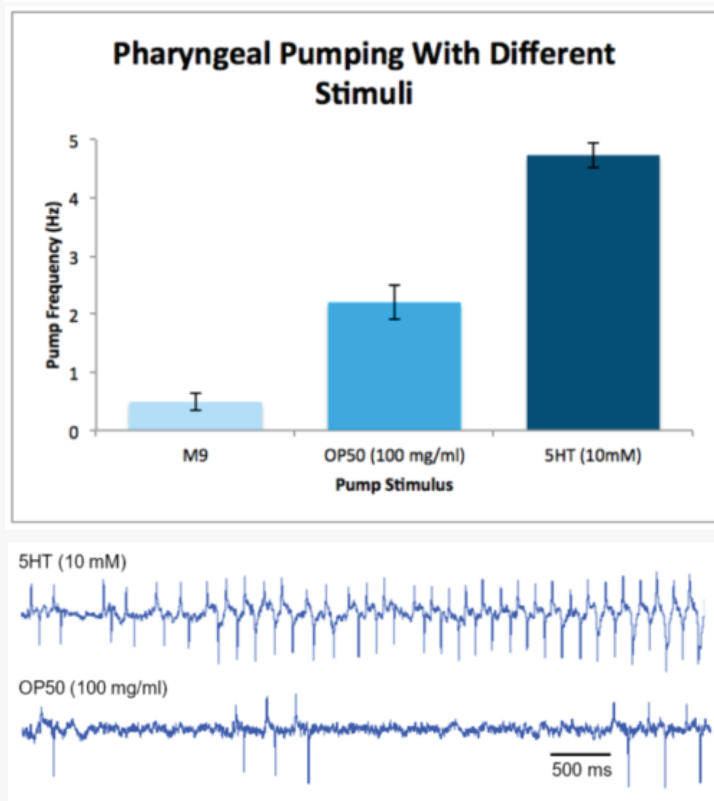
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with 100 mg/ml OP50, and EPG recordings were begun. Pharyngeal pumping frequency was measured from EPG recordings using the NemaMatrix ScreenChip system as described in the ScreenChip User Guide (7). Each EPG recording was 2 to 4 min in duration and the experiments were replicated in triplicate on different days.

RESULTS

The table and histogram below present pump frequencies for the three experimental groups (see Methods), with representative EPG recordings from the 5HT and OP50 groups.

	Pump Frequency (Hz)		
	M9 Control	10 mM 5HT Control	100 mg/ml OP50
Mean \pm S.E.M.	0.49 \pm 0.15	4.75 \pm 0.21	2.2 \pm 0.3*
n (worms)	38	56	38



Mean pump frequency was low (\sim 0.5 Hz) in the M9 control group and approached 5 Hz in the 10 mM 5HT group. Mean pump frequency in 100 mg/ml OP50 was intermediate. One-way ANOVA showed a significant effect of treatment ($F(2, 306) = 89.66, p = 1.1 \text{ E-}16$) with post hoc tests showing that all means differed significantly ($p < 0.01$; Tukey's HSD test). EPG recordings in 10 mM 5HT showed steady pumping with occasional, brief gaps whereas, in OP50, pumping occurred in bouts, with gaps between episodes of pumping

CONCLUSION

These experiments demonstrate that OP50 is a viable alternative to 5HT for stimulating pharyngeal pumping in the ScreenChip. No technical barriers were encountered. The mean pump frequency of 2.2 Hz obtained in 100 mg/ml OP50 ($\text{OD}_{600} = 3$) is similar to that obtained with OP50 concentrations of $\text{OD}_{600} = 2$ to 5. Thus, maximal pump frequency in OP50 may saturate at \sim 2-3 Hz, compared to \sim 5 Hz with 10 mM 5HT (Weeks et al., in preparation). To test compounds that inhibit pumping, 5HT may be preferable because the high baseline pump frequency allows inhibitory effects to be detected readily. For experiments investigating feeding and its regulation, using OP50 to stimulate pumping will provide a more naturalistic feeding stimulus.

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