



NemaMetrix

SEROTONIN-INDUCED PHARYNGEAL PUMPING IN *C. elegans*

USING THE SCREENCHIP SYSTEM

PURPOSE

The neuromodulator serotonin (5-hydroxytryptamine; 5HT) is often used to stimulate pharyngeal pumping in *C. elegans* and other nematodes (e.g., 1,2,3). To investigate the concentration-dependence of 5HT treatment on pump frequency measured in the ScreenChip platform, we tested the effects of 0, 2, 5 and 10 mM 5HT on N2 (wild type) adults on Day 1. Worms were pre-incubated in 5HT for 20 min to reach steady-state pump frequency, followed by recording electropharyngeograms (EPGs) from individual worms using the ScreenChip platform.

METHODS

C. Elegans synchronization and cultivation

Synchronized N2 *C. elegans* were obtained via a bleach synchronization procedure. 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 (Day 1) (4,5).

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. This stock was diluted 2:1 and 5:1 with M9 to obtain lower concentrations. All 5HT was used within 2 h of being prepared.

Recording electropharyngeograms (EPGs)

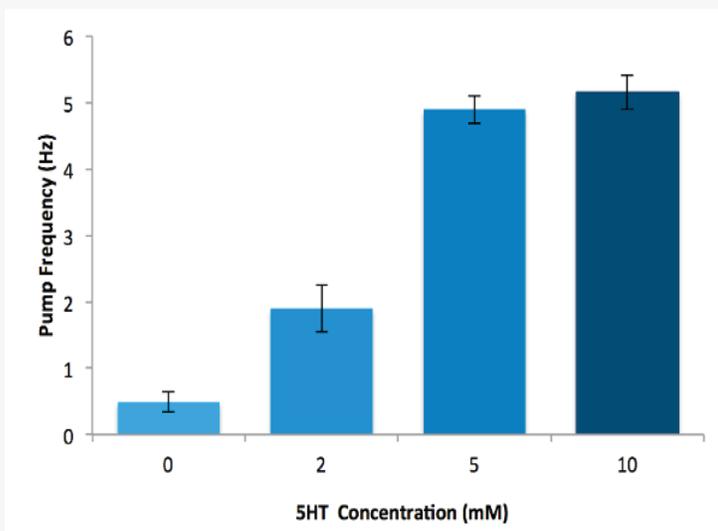
A glass pipette with M9 buffer was used to rinse Day1 adults from a plate. They were transferred to a 1.5 mL Eppendorf tube and centrifuged for 2 min at 6,000 RPM. The supernatant was removed and replaced with fresh M9 and the worms were spun down again. The supernatant was removed again and replaced with M9 containing the desired 5HT concentration. Worms were incubated for 20 min to activate pharyngeal pumping. Pump frequency was measured from EPG recordings using the NemaMetrix ScreenChip 100 system, as described in the ScreenChip User Guide (6). Each EPG recording was 2 to 4 min in duration and the experiment was replicated in triplicate on different days.



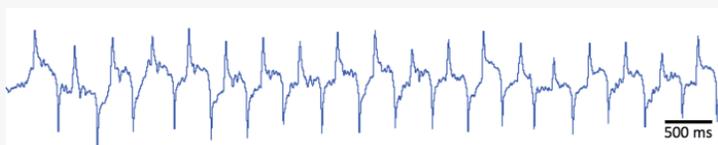
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RESULTS

The table and histogram below present pump frequency for the four experimental groups, with a representative EPG recording from the 10 mM group below.



[5HT], mM	0	2	5	10
	Pump Frequency (Hz)			
Mean ± S.E.M.	0.49 ± 0.15	1.9 ± 0.35	4.9 ± 0.21	5.16 ± 0.26
n (worms)	38	32	32	29



Mean pump frequency was <0.5 Hz in the absence of 5HT and exceeded 5 Hz in 10 mM 5HT. There was a statistically significant difference between groups as determined by one-way ANOVA ($F(3,127) = 95.13, p = 2.5E-32$). Post hoc tests showed that all means differed significantly ($p < 0.01$) except for the 5 mM and 10 mM 5HT groups (Tukey's HSD test).

CONCLUSION

As expected, serotonin (5HT) stimulated pharyngeal pumping in *C. elegans* N2 adults in the ScreenChip system, in a concentration-dependent manner. Depending on 5HT concentration, pump frequency varied by an order of magnitude, from ~ 0.5 Hz to ~ 5 Hz. Saturation of the effect was reached by 5 or 10 mM 5HT. In a previous study, 10 mM 5HT was used to elicit sustained pharyngeal pumping in *C. elegans*, against which the effects of inhibitory drugs were tested in 8-channel microfluidic EPG chips (3). We recommend the same testing protocol for the ScreenChip system.

REFERENCES

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