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Assessing behavioral toxicity of different substances using *Caenorhabditis elegans* as a biosensor

Online

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Introduction

Behavior, even in simple worms like *Caenorhabditis elegans*, depends upon integrated processes at the subcellular, cellular, and organismal level, and thus is susceptible to disruption by a broad spectrum of chemicals. This worm having even 80% homology with human genes offers a distinct advantage to be used as a biosensor for the evaluation of pesticide-induced environmental toxicity and risk monitoring [1]. Despite simple body structure *C. elegans* has an advanced chemosensory system that enables it to detect a wide variety of olfactory and gustatory cues. Much of its nervous system and more than 5% of its genes are devoted to the recognition of environmental chemicals [2]. Chemosensory cues can trigger chemotaxis, avoidance, and changes in overall locomotor activity. *C. elegans* as a biosensor also enables the detection of organism-level end points, for example feeding, reproduction, lifespan, and locomotion, the interaction of a chemical with multiple targets in an organism.

Aim of study

My goal was to construct a specialized platform that would enable the tracking of the nematode under high magnification. The nematode should be able to move freely throughout the Petri dish using chemosensation for navigation. The system should record and analyze the behavior of the nematode, its speed and position in relation to the place where it was applied to the Petri dish, to point where the substance was applied, and to point where the food was applied.

Method

I used my own worm tracker Matlab script to move the camera automatically to re-center the worm under the field of view during recording. The automated tracking system comprises a stereomicroscope (Olympus SZ11), a web camera to acquire worm videos, and a desktop PC running under Windows 10 (Fig 1).

***Figure 1.** A device recording locomotor activity and behavioral responses of nematode.*

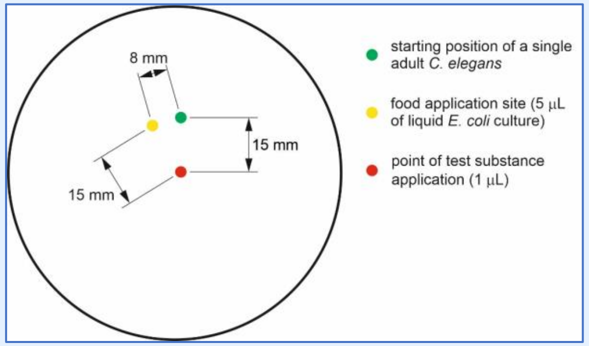

***Figure 2.** Experimental setup. The place where the nematode was applied is marked with a green dot. When the nematode was released from the water drop because of its evaporation/soaking, 1 µL of water (control) or substance was applied to the center of the dish (the area marked with a red dot). In the experiments with the presence of food, 10 minutes before the start of the experiment, an additional 5 µL of liquid E. coli culture was applied to the area marked with the yellow dot.*

I applied backlight techniques based on the transparency of both the container and media to illuminate the nematode. Backlight illumination obtains high-contrast images with dark *C. elegans* and a bright background. The radial Gaussian-shaped analyzed substance gradient was formed by adding 1 µL solution at the center of the plate just before the tracking (Fig. 2). Nearby I placed a drop of food (*E. coli* bacteria), in a strictly defined point for the system. The spatiotemporal track of each worm was reconstructed from the record of centroid locations and camera displacements. The instantaneous speed and trajectory were computed using the displacement of the centroid in successive samples. The tracking system recorded the worm's position, speed, and distance from the center of the plate and from the starting point. I used Fick's equation to estimate substance concentration in the surroundings the worm during the experiment.

Results

The results of preliminary studies show that my device and Petri dish setup are very useful in behavioral toxicology studies. Under the influence of certain substances in very low concentrations (ppm) in the environment, nematodes are unable to find the food in 60 min in contrast to control nematodes, which find food in no more than 5 min.

Conclusion

Mammalian models are very powerful but are expensive for high-throughput drug screens. The advantages of the *C. elegans* are mainly their low cost and ease of maintaining and breeding. Given the highly conserved neurological pathways between mammals and invertebrates, *C. elegans* has emerged as a powerful tool for behavioral toxicity but also for neurotoxic, and neuroprotective compound screening.

Reference

1. Saikia, S., Gupta, R., Pant, A., Pandey, R. (2014). Genetic revelation of hexavalent chromium toxicity using *Caenorhabditis elegans* as a biosensor. Journal of Exposure Science and Environmental Epidemiology 24, 180-184.
2. Bargmann, C.I. (2006). Chemosensation in *C. elegans*. WormBook, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.123.1, http://www.wormbook.org.