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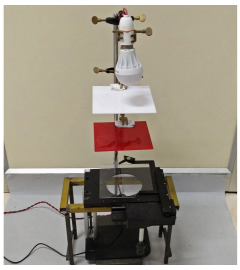
A Simple Method to Efficiently Record/ Capture Caenorhabditis elegans Locomotory Behaviours



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Abstract

Caenorhabditis elegans is an excellent model to study animal chemotaxis and thermotaxis behaviours. These nematodes have highly predictable behaviour pattern towards olfactory cues. A complex chemosensory information processing, based on both temporal and spacial cues, is involved in chemotaxis behaviour and can modify its behaviour towards attractants as well as repellents (Ward., 1973; Colbert et al., 1995; Troemel et al., 1997). In chemotaxis assay, worms show unsurpassed behaviour with a pattern of movement based on concentration gradient in the assay plate (Saeki et al., 2001; Iino et al., 2009). Such behavioural patterns are highly intriguing because they give better understanding on how various neuronal signalling elicit such pattern of behaviour and how factors such as past experience of the animal, mutations affecting neuronal function, modify them. (Brenner., 1974; de Bono et al., 1998). Hence, behavioural assays have critical role in elucidating the alterations in neuronal activities in *C. elegans.*

The standard chemotaxis assay estimates the movement pattern of C. elegans by tracking the course it takes in an agar plate containing a chemical gradient. This measurement requires recording the tracks over time in the plate (Buckingham et al., 2005; Yemini et al., 2011). Automated single worm tracker allows long term behavioural recording (Husson et al., 2005; Wang et al., 2013). The pattern of behaviour of animals in the assay plates shows there are significant alterations in patterns of movement like body bends and omega turns under experimental conditions. For a long term observation for such behaviour one needs to record the animals with least disturbances. Efficient recording often eliminates researcher's bias and makes it easy to re-evaluate the results if needed (Piere-Shimoura et al., 1999; Hardaker LA et al., 2001; Baek et al., 2002). These recorded videos can be later processed in ImageJ for measuring the patterns. Though these recording can be done using a simple dissection microscopic system with camera, there is a major limitation that the light source is very close to the worm making series of artefacts in its behaviour,

Here we report a simple setup to manually record and count these behavioural changes in worms. In this study we measured basal slowing response and enhanced slowing responses, the two different locomotory changes in response to food. Neuronal circuitry underlying these locomotory changes involves dopamine and serotonin (Sawin et al 2000).

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Attachments

FILE Video-1.mp4 10.4MB

FILE Video-2.mp4 11.5MB

Materials

Dino-Lite microscope (Model AM4113T-GFBW), Dinocapture2.0 software (available online), Dino microscope stand (RK-10), Burette stand and clamps, Acrylic sheets (transparent, red and white), Metal stand (Steel made; Length 30cm X Breadth 15cm X Height 30cm), Manual stage-Olympus; Model No: IX2-KSP-9C13573, Philips 7W LED bulb, Computer with 2GB RAM, N2 Bristol (strain obtained from CGC, Minnesota, USA), Petri dishes (60mm), NG Media.

Setting up the camera

1 The skeleton of this system is a burette stand and clamps to hold various parts (Fig.1a). For video capturing we used Dino-Lite digital microscope (Model no AM4113T-GFBW) with USB connector and its software module Dinocapure2.0 (Fig.1b) to record the data. Dino-Lite digital microscope was fixed on a table top stand (Dino-Lite RK-10) enables smooth focus adjustment and quick vertical movements (Fig.1b). An X-Y stage was placed over on a custom-made metal frame (Fig.1c). Petri dish containing the worms were placed on a transparent acrylic sheet placed on this XY stage (if the stage has a universal holder, petri dish can be directly held on the stage).

2 As illumination source, 7 W led white light was used (Fig.1d). To enhance the image contrast as well as uniform illumination we used white and a red acrylic sheets (Fig.1e). The gap between LED light source and the white acrylic sheet was adjusted between 4-7 cm; adjusted based on the required intensity of light to visualize the animals clearly. The distance between white and red acrylic sheets was 7-15 cm (Fig.1e). Parallel alignment of both these sheets found to be crucial for the set up.

3 The USB microscope was connected to a computer though USB cable. All recording of the video was done using the Dino-Lite software module. Using this system, we tested the animal behaviour in the presence and absence of food and could establish significant variation in body bends (Fig.1h).