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A Simple Light Stimulation of *Caenorhabditis elegans*

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Abstract

Response via noxious stimulus can be an important indicator of sensory neuron function and overall health of an organism. If the stimulation is quick and simple, and the animal can be rescued afterwards, such method not only allows for assays pertaining to changed sensory ability after various treatments, but also increased strength of statistical relationships established. This protocol demonstrates a stimulation assay in *Caenorhabditis elegans*, using blue light from common laboratory equipment: the fluorescent microscope. The nematode detects blue light using a set of amphid ciliary sensory neurons, and blue light is detrimental to its overall health after a prolonged exposure. However, under brief exposure, blue light stimulation provides a rapid and easy method for quantifying sensory functions and health without harming the animal.

Keywords

Caenorhabditis elegans; stimulation; threshold; blue light

INTRODUCTION

Caenorhabditis elegans is a free-living nematode, often found in soil. It is the first multicellular organism with a complete genome sequence, and over 80% of human genes have orthologs in the *C. elegans* genome (Lai et al, 2000). The ease of genetic manipulation allows for generation of transgenics, overexpressors, knock-outs, and functional null deletions. The transparency of *C. elegans* enables ease of imaging of physiologic processes, and fluorescent proteins and biochemical dyes have been used to visualize cells, cellular processes, and biologic compounds. *C. elegans* is also the first model organism with a complete connectome, a map of synapses throughout the entire animal (White et al, 1986). Its neurotransmitter signaling is conserved, and many common signaling pathways, such as dopamine, serotonin, and various neuropeptides, have been well-characterized in the nematode (Sawin et al, 2000; Li et al, 1999). Short life-span and ease of culturing in a laboratory setting, in addition to aforementioned advantages, makes *C. elegans* a powerful neurogenetic tool, suitable for studying and modeling human neurologic diseases and syndromes.

Two main types of sensory neurons mediate *C. elegans* response to stimulus: mechanosensory and chemosensory. A unique feature in *C. elegans* nervous system is that

the sensory neurons are the only ciliated cell type. The ciliary sensory neurons are polymorphic, ranging from rodlike to winged, and are polymodal, each enabling the nematode to detect a variety of stimuli (Inglis et al, 2007).

Response to noxious stimulus has been used to describe many *C. elegans* behaviors and physiology, such as elevated threshold level during lethargus, neurotransmitter modulation of avoidance behaviors, and longevity (Raizen et al, 2008; Baidya et al, 2013; Solomon et al, 2004). The mediation of many behaviors are neurologically redundant, due to the polymodal nature of sensory neurons in *C. elegans* (Inglis et al, 2007). This allows for multiple ways to stimulate the nematode to check the integrity of its sensory nervous system and its ability to respond accordingly.

Described in the following is a stimulation method using blue light. Sensitivity to light was once thought to be irrelevant in *C. elegans*, as its natural habitat is in the soil, where there is minimal sun exposure. However, it has been recently shown that light, especially in the 470–350 nm spectrum, elicits dose-dependent, negative phototactic behavior in *C. elegans* (Ward et al, 2008). Further evidence as its noxious effects on the nematode is demonstrated by prolonged exposure to blue light, which leads to paralysis, cessation of feeding, and death. Photosensitivity has been shown to be mediated by ASJ, AWB, ASK, and ASH, all of which are amphid ciliary chemosensory neurons (Ward et al, 2008). These neurons also mediate dauer formation and recovery, avoidance of volatile chemicals, and social feeding, among other behaviors (Bargmann, 2006). Therefore, it follows that deficient avoidance behavior to blue light could indicate dysfunctions of the four amphid neurons, their associated behaviors, or dysfunctions of locomotive mechanisms. With ease of exposure, common availability of blue light in a laboratory, redundant circuitry in the sensory nervous system, blue light stimulation is a simple and easy method to assess the sensory abilities of *C. elegans*.

BASIC PROTOCOL 1: Quantification of Stimulation Threshold in *C. elegans* Using Blue Light from Fluorescent Dissecting Microscope

This method provides a simple way to stimulate the nematode and quantify its threshold of stimulation without sacrificing the animal. The stimulation and quantification steps are done under the fluorescent dissecting microscope. The absence of noxious chemicals and use of common laboratory equipment may make this method more accessible than other stimulation methods.

Materials

C. elegans (hermaphrodites or males; developmental stage of interest)

NGM agar in 30 or 60 mm petri dish (see recipe)

OP50 in LB broth (see recipe)

37°C incubator (Lab-Line Instruments, Inc. model no. 150)

Paper towel

Platinum wire-pick

A dissecting microscope with fluorescence (Leica MZ16 FA; Arc Lamp Power Supply, HBO 100 DC IGN, cat. #990014)

Protocol

1. Seed fresh NGM plates with OP50, by making a dot (~1 cm in diameter) in the center of the agar.
2. Allow 10–15 minutes for the bacterial broth to be soaked up by the agar.
3. Flip the seeded agar plates upside down, and incubate the seeded plates at 37°C for 3–4 hours.
4. After the incubation, cool the plates until they reach room temperature.
5. Transfer a single nematode onto the cooled NGM plate.
6. Wipe any residual moisture from the petri dish cover using a paper towel.
7. Turn on the fluorescence lamp of the microscope. Allow the bulb to warm up, and make sure the fluorescence shutter is closed.
8. With the lid closed, place the NGM plate with the worm sample under the fluorescent dissecting microscope.
9. Adjust the diaphragm and magnification, appropriate for observing the behavior of interest.
10. Open the fluorescence shutter.
11. Quantify or observe the behavior of interest.

REAGENTS AND SOLUTIONS

NGM agar (1L)

1. Add 3 g NaCl (Fisher Scientific, cat. BP358-212), 2.5 g peptone (BD, cat. 211820), 17 g granulated agar (BD, cat. 214030), and ~975 mL ddH₂O into a 2 L Erlenmeyer flask.
2. Autoclave for 45 minutes on liquid cycle.
3. After cooling to 50°C, add 1 mL cholesterol in ethanol (5 mg/mL) (Sigma, cat. C8667), 1 mL 1 M MgSO₄ (Fisher Scientific, cat. BP213-1), 1 mL 1 M KPO₄ (pH 6) (Fisher Scientific, cat. P285-3), 1 mL streptomycin sulfate (100 mg/mL) (Sigma, cat. S9137), and 1.25 mL Nystatin (10,000 units/mL in DPBS) (Sigma, cat. N1638).
4. Aliquot 10 mL per 60 mm plate, or 4 mL per 30 mm plate.

LB broth (1L)

1. Add 10 g tryptone (Sigma, cat. T9410), 5 g yeast extract (Sigma, cat. Y1625), 10 g NaCl, and 1 L ddH₂O into a 2 L Erlenmeyer flask.

2. Adjust pH 7.0 using NaOH (Fisher Scientific, S318)
3. Autoclave for 45 minutes on liquid cycle.
4. Aliquot desired volume in a conical tube and inoculate with OP50.
5. Place in shaker and incubate at 37°C overnight.

COMMENTARY

Background Information

This is a modified version of the protocol first outlined by Ward et al in 2008. Blue light response in *C. elegans* has been shown to be mediated only by select amphid sensory neurons, making focusing the light only to the head region unnecessary. Blue light is a noxious stimulus; therefore, upon stimulation, the nematode will exhibit typical avoidance behaviors, such as acceleration and reversal. If stimulated during lethargus, the worm will awake and move accordingly to avoid the blue light.

A potential application of the blue light stimulation is shown in Figure 1. To observe whether chronically exposing the nematode to manganese would alter its threshold of stimulation, wild-type N2 was grown on MYOB agar supplemented with 1.5 mM NaCl or 1 mM Mn, as well as on untreated MYOB agar. During L4, worms grown on each condition were singled and allowed to enter L4 lethargus, a sleep-like state that follows each of the four nematode larval periods. Afterwards, threshold of stimulation was quantified using the blue light method. Worms grown on MYOB and MYOB with 1.5 mM NaCl showed no difference in response time to blue light. Worms grown on 1 mM Mn seemed to show shorter response time to blue light, suggesting that manganese, and not mere osmolar effects, decreased the threshold of stimulation during L4 lethargus.

Troubleshooting

It is critical to minimize the presence of other stimuli, such as mechanical vibrations, temperature variations, and air flow, and there is minimal absorption and scattering of the light. Keeping the petri dish lid closed during the assay helps to remove the worms' exposure to air, which does stimulate them to move. Residual moisture on the petri dish cover was found to be the most common cause of light scattering. It is also important to standardize the intensity of the light source. This can be done by using the same magnification and diaphragm diameter for each sample. If the worm is stimulated prior to the light exposure, allowing for a 10–15 minute rest will return it to its basal feeding and locomotive rates.

Anticipated Results

As blue light is a noxious stimulus, the nematode will exhibit typical avoidance behaviors when exposed to it. If shone during lethargus, the worm will awake. If shone during wake periods, the worm will accelerate, reverse, or make an omega turn. Prolonged exposure to blue light will result in cessation of feeding and ultimately death of the nematode.

Time Considerations

The most laboring part of this protocol is preparing the seeded NGM plates, which will take about four hours. Handling of the nematode would greatly depend on the experience of the experimenter. On average, an average worm handler can transfer a single worm in a few seconds.

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Figure 1.

Blue-light stimulation of N2 during L4 lethargus. n = 7 trials for each growth condition. The y-axis represents the length of time until movement, defined as any change in the animal's shape viewed under the microscope. Error bars show standard error. Statistical significance was assessed using ANOVA.