Neurophysiological methods in *C. elegans*: an introduction*

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Abstract

The simple and well-defined structure of the *C. elegans* nervous system has made it an attractive model for studying the neural and genetic basis of behavior. However, the wider use physiological methods for monitoring neural activity *in vivo* or determining the effects of specific ion channels on neuronal function has been a relatively recent development. This chapter presents a compendium of protocols and technical reports on the current state of the art in *C. elegans* electrophysiology and neuroimaging. These include methods for calcium imaging in intact animals, in situ electrical recording from neurons and muscle cells, and *in vitro* recording from cultured neurons and oocytes.

Traditionally, neurobiological studies in *C. elegans* have relied almost exclusively on behavioral genetics. Mutants with abnormalities in one or more of the many well-defined behavioral assays have been used to identify genes with critical functions in such processes as neural development, sensory perception, and motor activity. Because of the ease of transgenesis and the simplicity of the *C. elegans* nervous system, it is usually possible to identify the cellular focus of a particular mutant gene through cell-specific rescue, and such experiments, combined with laser ablation studies, can be used to identify neurons with specific roles in particular behaviors. Consequently, behavioral assays often can serve as indirect measures of the activity of particular neurons and muscle cells.

However, the use of behavioral assays alone has important limitations as a way to study the molecular and cellular basis of behavior. For example, to understand mechanisms of behavior in detail, it is necessary not only to identify neurons that are required for that behavior, but also to understand how their temporal patterns of activity correlate with associated behavioral events. Likewise, to understand how a particular gene influences a behavior, it is necessary to determine how it affects the functional properties of the individual neurons involved in generating that behavior. It is often desirable to specifically determine the effect of a gene on a particular ionic conductance in a given neuron or muscle cell, or even to reconstitute the activity of a particular ion channel in a heterologous system.

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Various techniques have been developed by physiologists to address these questions. Some of these can be straightforwardly adapted for studies in *C. elegans*, while the application others in nematodes have required significant technical breakthroughs. Below, some of the important advantages and disadvantages of particular physiological approaches are discussed in the context of their most appropriate applications. Detailed presentations of these individual methods can be found in the other chapters of this section.

1. Electrophysiology

The standard approach for monitoring neural activity in most animals involves recording electrically from neurons. These techniques have been difficult to apply in *C. elegans* for at least two reasons. First, *C. elegans*, like other nematodes, has a hydrostatic skeleton; its body shape and structure are provided by a tough proteinaceous cuticle enclosing a highly pressurized pseudocoelomic fluid. The cuticle presents a formidable barrier to a recording electrode, and an animal must be dissected carefully to avoid damaging the preparation through release of hydrostatic pressure. Because such dissections necessarily disrupt the hydrostatic skeleton, making electrical recordings from behaving animals is not really feasible. Second, *C. elegans* neurons are quite small, with cell bodies only 2-3 microns in diameter. Thus, even when a *C. elegans* neuron has been exposed through dissection, making intracellular recordings requires a great deal of skill.

Despite these challenges, several electrophysiological preparations have been developed for *C. elegans* (e.g., Goodman et al., 1998; Raizen and Avery, 1994; Rogers et al., 2001); see following chapters by Holden-Dye and Goodman). These preparations have proven invaluable for addressing a variety of neurobiological questions, as electrical recording remains the only feasible way to measure a wide range of physiological parameters in an *in vivo* context. For example, a diverse array of ionic conductances have been measured in *C. elegans* neurons and neuromuscular junctions, including those mediated by nicotinic acetylcholine receptors (Richmond and Jorgensen, 1999), GABA receptors (Richmond and Jorgensen, 1999), glutamate receptors (Brockie et al., 2001), voltage-gated calcium channels (Lee et al., 1997), and mechanosensory transduction channels (O'Hagan et al., 2005).

2. Optical imaging

A less invasive approach to monitoring neural activity *in vivo* is through the use of fluorescent optical indicators. Thus far, the most effective means of optically imaging neuronal dynamics is using genetically-encoded calcium probes such as cameleon or GCaMP (Miyawaki et al., 1997; Nakai et al., 2001). Since nematode neurons lack voltage-gated sodium channels, calcium entry through voltage-gated channels is thought to play a key role in the neuronal excitability. Calcium transients accompanying neuronal activity have been detected in the cell bodies, and in some cases the processes, of many neurons (Hilliard et al., 2005; Kerr et al., 2000; Kimura et al., 2004; Shyn et al., 2003; Suzuki et al., 2003).

Calcium imaging has several attractive features as a way to monitor neural activity. In particular, since *C. elegans* are transparent, optical recordings can be made without the need for dissection. Thus, it is possible to record neural activity in intact, even behaving animals. In addition, because the *C. elegans* nervous system is compact, it is possible in principle to image from multiple neurons simultaneously. Such multichannel recordings make it possible to identify temporal correlations in neural activity that can provide insight into the mechanisms of neural circuit function. The major limitation of this approach is that calcium provides only a single readout of neural activity, and does not allow one to measure individual ionic conductances or detect sub-threshold changes in membrane potential. Additionally, the temporal resolution of optical imaging methods are limited by camera speed and by the kinetic properties of the probe.

3. *In vitro* physiology

To obtain detailed information about specific ionic conductances and their dependence on individual gene products, it is often desirable to use *in vitro* methods, Despite the considerable evolutionary divergence between nematodes and vertebrates, many *C. elegans* ion channels and receptors have been expressed heterologously in *Xenopus* oocytes (Squire et al., 1995; Weinshenker et al., 1999; Bianchi et al., 2004) or mammalian fibroblasts (Zhang et al., 1997) and shown functional activity. In addition, methods have recently been developed for *C. elegans* primary cell culture (Christiansen et al., 2002; Zhang et al., 2002), which has made it possible to compare the physiological properties of wild-type and mutant cells *in vitro*.

These methods have several important applications in *C. elegans* neurobiology. For example, physiological studies of mutants can indicate only whether a given gene product is necessary for a particular neural function. By using heterologous expression to reconstitute a functional complex in a non-neuronal cell, it is possible to determine which proteins are sufficient for the activity of a particular receptor or channel. Likewise, by using cell culture, it is possible to determine the cell-autonomous effect of mutating a particular channel or receptor gene in the absence of indirect developmental or circuit-related effects related to gene function in other cell types. Finally, for studies in which the *in vivo* context is not critical, recording from an oocyte or cultured cell provides less of technical challenge for electrophysiology than recording *in situ* from a dissected animal.

In conclusion, it should be noted that variations on, or combinations of the methods presented here can be applied to different experimental situations. For example, the calcium imaging methods described in the *in vivo* neuroimaging section can also be used with dissected animals or cultured cells (Bianchi et al., 2004). Likewise, optical indicators of physiological parameters other than calcium can be used *in vitro* and *in vivo* (Samuel et al., 2003). With the development of novel neural indicator molecules as well as new techniques for dissecting and manipulating nematodes, physiological methods are likely to play an ever-increasing role in studies of *C. elegans* nervous system function and behavior.

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