The quest for action potentials in *C. elegans* neurons hits a plateau

Shawn R Lockery & Miriam B Goodman

The small size and high resistance of *C. elegans* neurons makes them sensitive to the random opening of single ion channels, probably rendering codes that are based on classical, all-or-none action potentials unworkable. The recent discovery in *C. elegans* of a special class of regenerative events known as plateau potentials introduces the possibility of digital neural codes. Such codes would solve the problem of representing information in nervous systems in which action potentials are unreliable.

The publication in 1986 of an essentially complete wiring diagram of the *C. elegans* nervous system[1] raised the prospect of the first comprehensive account of the behavior of an entire organism. It quickly became apparent, however, that a wiring diagram by itself is insufficient to explain behavior, even for an organism as simple as a nematode. Clearly, one also needs to know the intrinsic electrical properties of the neurons. Do they act as passive or active nodes? If active, do they fire all-or-none action potentials, graded regenerative responses or something else? With the introduction of specialized electrophysiological methods for patch-clamping *C. elegans* neurons[2–5], several laboratories are now addressing these questions directly. A recent study[6] reported the surprising finding that the answer, at least in some *C. elegans* neurons, is something else.

Recording *in situ* from the motor neuron class RMD, these researchers noted a distinctive regenerative response to current injection. A brief pulse of positive current in an RMD neuron (or a puff of glutamate) elicited a depolarization of more than 30 mV that was stable for at least 1 min (**Fig. 1a**) but could be terminated by a brief pulse of negative current. Thus, RMD neurons have two stable resting potentials, one near –70 mV and one near –35 mV. Notably, shifts between the two resting potentials were also observed to occur spontaneously, indicating that these events probably happen naturally in RMD neurons.

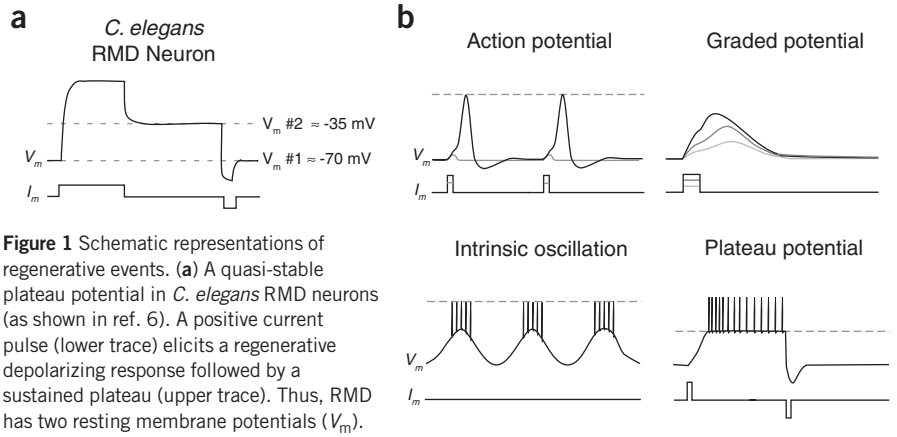

Figure 1 Schematic representations of regenerative events. (**a**) A quasi-stable plateau potential in *C. elegans* RMD neurons (as shown in ref. 6). A positive current pulse (lower trace) elicits a regenerative depolarizing response followed by a sustained plateau (upper trace). Thus, RMD has two resting membrane potentials (V_m). The plateau can be terminated by a negative current pulse (after Mellem *et al.* 2008). (**b**) Four common types of regenerative neuronal activity. Intrinsic oscillations and plateau potentials are frequently accompanied by trains or bursts of spikes in neurons capable of firing action potentials (vertical lines). Dashed lines indicate amplitudes of all-or-none events.

Electrophysiologists have described four main types of regenerative events: action potentials, graded potentials, intrinsic oscillations and plateau potentials (**Fig. 1b**). The defining features of action potentials are well known; they are all-or-none depolarizations with a stereotypical waveform that is independent of the amplitude and waveform of the triggering stimulus. The defining features of other types of regenerative events may be less well known. Graded potentials resemble action potentials, but their amplitude and waveform are sensitive to the amplitude and waveform of the stimulus. Intrinsic oscillations, such as those that underlie endogenous bursting, are slow, cyclic alterations in membrane potential that are caused by antagonistic voltage-dependent currents. Plateau potentials are prolonged, all-or-none depolarizations that can be triggered and terminated by brief positive- and negative-current pulses, respectively[7,8].

Regenerative activity has been seen before in nematode neurons. In *Ascaris*, motor neurons fire graded potentials in response to the offset of hyperpolarizing current pulses[9]. Inhibitory motor neurons show slow oscillations that are probably intrinsic[10], as do certain ventral cord interneurons[11,12]. There is also indirect evidence of Ca^{2+}-dependent action potentials in the ventral nerve cord[13]. In *C. elegans*, depolarizing current pulses in chemosensory

Shawn R Lockery is at the Institute of Neuroscience, University of Oregon, Eugene, Oregon, USA. Miriam B Goodman is in the Department of Molecular and Cellular Physiology, Stanford University, Stanford, California, USA. e-mail: shawn@uoregon.edu

neurons elicits active responses that outlast the stimulus pulse[2]. The events recorded by the above-mentioned study[6] fit the criteria of plateau potentials perfectly in that they are long-lasting, all-or-none events that can be terminated by a negative-current pulse. In addition, the genetic manipulations and ion-substitution experiments performed in that study[6] suggest that RMD plateau potentials depend on a current that is carried mainly by Na^+ and Ca^{2+} ions, which is consistent with the biophysical mechanisms of plateau potentials in other organisms[14–19].

The discovery of plateau potentials in *C. elegans* neurons is an important development, for it greatly expands the computational repertoire of the *C. elegans* nervous system. Plateau potentials are the biological equivalents of Schmitt triggers, which have many interesting applications, including oscillators, timers and flip-flop elements. Thus, it is not surprising that plateau potentials have been reported in a wide range of vertebrate and invertebrate organisms[20–26], where they have been implicated in such functions as pattern generation and short-term memory. At present, almost nothing is known about either function in *C. elegans*, despite long-standing behavioral evidence of multiple pattern generators[27–29] and several forms of associative and nonassociative learning[30].

The findings of the new study[6] raise many new questions that the field will now be keen to answer. Do most *C. elegans* neurons exhibit plateau potentials? If not, which functional subsets of neurons exhibit them and what purposes do they serve? Is plateau behavior an intrinsic property of *C. elegans* neurons or does it arise, at least in part, through synaptic interactions? The latter is a possibility because at least some *C. elegans* neurons appear to release neurotransmitter at the resting potential[31,32]. What ion channels are required for plateau potentials and how might they be regulated by learning and other forms of experience?

The quest for action potentials in *C. elegans* neurons is really just beginning. To date, current-clamp recordings have been published for only 4 of the 118 anatomical neuron classes in *C. elegans* hermaphrodites[2,6,33,34]; thus, action potentials may yet be found in some classes of neurons. But if action potentials turn out to be the main currency of information transfer in *C. elegans*, it may come as something of a surprise, at least to theoreticians who have considered the signaling properties of neurons near what might be termed the small-cell limit. At this spatial scale, there may be only a few tens or hundreds of sodium and potassium channels in an entire neuron and input resistance is so high that the opening of a single sodium channel can trigger an action potential, or what is left of one. Many *C. elegans* neurons are likely to be nearly isopotential[2] and reducing isopotential model neurons to the small-cell limit has profound effects on signaling. For example, when a Hodgkin-Huxley model is reduced, an otherwise silent neuron fires tonically at average rates of tens of spikes per second[35,36]. Moreover, firing is so irregular that the s.d. of interspike intervals approaches the size of the intervals themselves. Together, these two effects render coding schemes that are based on firing rate and spike timing essentially unworkable. Additional problems arise when one considers spike propagation along thin axons (diameter < 500 nm), including spurious or deleted spikes and the redistribution of spike arrival times[37].

Evidence that *C. elegans* neurons operate at the small-cell limit is already quite strong. Measured input resistances in medium-sized *C. elegans* neurons are on the order of Gohms[2] and the true input resistance is probably much higher when one takes into account the Gohm order leak of the seal resistance in a whole-cell patch-clamp experiment. In this range, the opening of a single ion channel can cause voltage fluctuations on the order of millivolts to tens of millivolts. Process diameters of most *C. elegans* neurons are in the range of 100–200 nm[38] and it is estimated that chemosensory neurons, whose size and morphology are typical of most neurons in the worm's head, have only about 50 voltage-dependent calcium channels[2], the probable carrier of inward current in *C. elegans*[39]. Coding schemes that are based on the amplitude or duration of graded potentials are also probably unworkable, as these parameters would also be strongly sensitive to the random behavior of small numbers of highly effective channels. Thus, however *C. elegans* neurons may code information, it is probably different from the way it is done in the nervous systems with which we are currently more familiar and that have much larger neurons.

Perhaps the most important aspect of the finding of plateau potentials in *C. elegans* neurons is the prospect of an elegant solution to the small-cell coding problem: how is information represented when both action potentials and graded potentials are unreliable? In *C. elegans*, the small cell problem is particularly acute because there are at most six neurons in any functional class, a fact that eliminates averaging across multiple neurons as a remedy. Action potentials and graded potentials support analog coding schemes, firing rate and spike timing as being analog quantities, whereas plateau potentials might support a digital coding scheme in which neuronal state (depolarized or hyperpolarized) stores the sign of the most recent synaptic input. Digital codes are, of course, famous for their immunity to noise, spurious voltage offsets and other probable afflictions of small-neuron networks. There are already tantalizing hints that the direction of locomotion in *C. elegans* (forward versus reverse) is coded digitally[40]. If it turns out that plateau potentials are the dominant mode of electrical signaling in *C. elegans*, we might soon be getting our first look at a digital nervous system.

1. White, J.G., Southgate, E., Thomson, J.N. & Brenner, S. *Phil. Trans. R. Soc. Lond. B* **314**, 1–340 (1986).
2. Goodman, M.B., Hall, D.H., Avery, L. & Lockery, S.R. *Neuron* **20**, 763–772 (1998).
3. Brockie, P.J., Mellem, J.E., Hills, T., Madsen, D.M. & Maricq, A.V. *Neuron* **31**, 617–630 (2001).
4. Christensen, M. *et al. Neuron* **33**, 503–514 (2002).
5. Nickell, W.T., Pun, R.Y., Bargmann, C.I. & Kleene, S.J. *J. Membr. Biol.* **189**, 55–66 (2002).
6. Mellem, J.E., Brockie, P.J., Madsen, D.M. & Maricq, A.V. *Nat. Neurosci.* **11**, 865–867 (2008).
7. Marder, E. *Curr. Biol.* **1**, 326–327 (1991).
8. Russell, D.F. & Hartline, D.K. *J. Neurophysiol.* **48**, 914–937 (1982).
9. Davis, R.E. & Stretton, A.O.W. *J. Neurosci.* **9**, 415–425 (1989).
10. Angstadt, J.D. & Stretton, A.O.W. *J. Comp. Physiol. [A]* **166**, 165–177 (1989).
11. Angstadt, J.D., Donmoyer, J.E. & Stretton, A.O. *J. Comp. Neurol.* **284**, 374–388 (1989).
12. Holden-Dye, L. & Walker, R.J. *Parasitology* **108**, 81–87 (1994).
13. Davis, R.E. & Stretton, A.O.W. *J. Comp. Physiol. [A]* **171**, 17–28 (1992).
14. Lee, C.R. & Tepper, J.M. *J. Neurosci.* **27**, 6531–6541 (2007).
15. Lo, F.S., Ziburkus, J. & Guido, W. *J. Neurophysiol.* **87**, 1175–1185 (2002).
16. Otsuka, T., Abe, T., Tsukagawa, T. & Song, W.J. *J. Neurophysiol.* **92**, 255–264 (2004).
17. Simon, M., Perrier, J.F. & Hounsgaard, J. *Eur. J. Neurosci.* **18**, 258–266 (2003).
18. Amat, C., Lapied, B., French, A.S. & Hue, B. *J. Neurophysiol.* **80**, 2718–2726 (1998).
19. Zhang, B. & Harris-Warrick, R.M. *J. Neurophysiol.* **74**, 1929–1937 (1995).
20. Mercer, A.R., Kloppenburg, P. & Hildebrand, J.G. *J. Neurophysiol.* **93**, 1949–1958 (2005).
21. Derjean, D., Bertrand, S., Nagy, F. & Shefchyk, S.J. *J. Physiol. (Lond.)* **563**, 583–596 (2005).
22. Angstadt, J.D. & Choo, J.J. *J. Neurophysiol.* **76**, 1491–1502 (1996).
23. Di Prisco, G.V., Pearlstein, E., Robitaille, R. & Dubuc, R. *Science* **278**, 1122–1125 (1997).
24. Susswein, A.J., Hurwitz, I., Thorne, R., Byrne, J.H. & Baxter, D.A. *J. Neurophysiol.* **87**, 2307–2323 (2002).
25. Sierra, F., Comas, V., Buno, W. & Macadar, O. *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.* **191**, 1–11 (2004).
26. Scroggs, R.S. & Anderson, E.G. *Brain Res.* **485**, 391–395 (1989).
27. Niebur, E. & Erdos, P. *Biophys. J.* **60**, 1132–1146 (1991).
28. Thomas, J.H. *Genetics* **124**, 855–872 (1990).
29. Hart, A.C., Sims, S. & Kaplan, J.M. *Nature* **378**, 82–85 (1995).
30. Rankin, C.H. *Curr. Biol.* **14**, R617–R618 (2004).
31. Chalasani, S.H. *et al. Nature* **450**, 63–70 (2007).
32. Suzuki, H. *et al. Nature* **454**, 114–117 (2008).
33. O'Hagan, R., Chalfie, M. & Goodman, M.B. *Nat. Neurosci.* **8**, 43–50 (2005).
34. Ramot, D., Macinnis, B.L. & Goodman, M.B. *Nat Neurosci.* **11**, 908–915 (2008).
35. Strassberg, A.F. & DeFelice, L.J. *Neural Comput.* **5**, 843–855 (1993).
36. Faisal, A.A., White, J.A. & Laughlin, S.B. *Curr. Biol.* **15**, 1143–1149 (2005).
37. Faisal, A.A. & Laughlin, S.B. *PLoS Comput. Biol.* **3**, e79 (2007).
38. Hall, D.H. & Altun, Z. *C. elegans Atlas* (Cold Spring Harbor Press, Woodbury, New York, 2008).
39. Bargmann, C.I. *Science* **282**, 2028–2033 (1998).
40. Chronis, N., Zimmer, M. & Bargmann, C.I. *Nat. Methods* **4**, 727–731 (2007).