BRIEF COMMUNICATIONS

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Action potentials contribute to neuronal signaling in *C. elegans*

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Small, high-impedance neurons with short processes, similar to those found in the soil nematode *Caenorhabditis elegans*, are predicted to transmit electrical signals by passive propagation. However, we have found that certain neurons in *C. elegans* fire regenerative action potentials. These neurons resembled Schmitt triggers, as their potential state appears to be bistable. Transitions between up and down states could be triggered by application of the neurotransmitter glutamate or brief current pulses.

The nematode *C. elegans* is widely used for genetic studies of nervous system development and function. One attractive feature of *C. elegans*, a self-fertilizing hermaphrodite organism with 302 neurons, is the prospect of gaining a molecular understanding of how neural circuits control behavior[1]. In most of the vertebrate nervous system, information is coded by the frequency of action potentials, regenerative all-or-none changes in the membrane potential that allow the transmission of information over long distances without the decrease in information content that would occur with passive propagation. Although many molecules are conserved between *C. elegans* and vertebrate nervous systems, predicted channel proteins that would contribute to Na^+-dependent action potentials have not been identified[2], suggesting that *C. elegans* neurons may transmit information by simple passive propagation. This notion is supported by an *in vivo* electrophysiological study that found select neurons to be isopotential, with no evidence of classical action potentials, suggesting passive signal propagation along high-impedance neurons[3]. In addition, electrical recordings have not revealed action potentials in the parasitic nematode *Ascaris suum*[4]. On the other hand, there is indirect evidence that *C. elegans* neurons may have intrinsic membrane properties that allow the generation of action potentials[5]. Moreover, action potentials in neurons and muscles can be generated by the activation of voltage-gated calcium channels, and several studies have suggested the possibility of active signaling in *C. elegans* muscles[5,6] and graded active responses in *Ascaris*[7].

We found that at least one class of neurons in *C. elegans* fires regenerative action potentials that can lead to long-lived changes in membrane potential. *C. elegans* RMD class interneurons, which synapse with interneurons and muscle cells, and AVA command interneurons, which synapse with motor neurons, primarily contribute to the control of head movements and locomotion[8]. These neurons express ionotropic glutamate receptors, including the AMPA receptor subunit GLR-1 (ref. 9). Using previously described patch-clamp recording techniques[10], we recorded from a neuron immediately adjacent to AVA in transgenic worms that expressed a GFP transgene under control of the *glr-1* promoter. On the basis of position and GFP expression, we believed that this neuron was RMD. In 14 out of 16 RMD neurons, we found that the voltage response to depolarizing current ramps was linear from approximately –80 mV to –60 mV, but the voltage response then became regenerative, leading to a solitary action potential, with no bursting (**Fig. 1a**). In contrast, we never observed action potentials in AVA neurons ($n = 10$; **Fig. 1b**). We also found that small, depolarizing current steps were sufficient to generate long-lived action potentials in RMD neurons (**Fig. 1c**). On cessation of the current step, the voltage relaxed to a new steady-state value of approximately –10 mV, in marked contrast to the initial resting potential of approximately –73 mV (**Fig. 1c**). We found bistable potentials associated with 54 out of 98 RMD action potentials. In

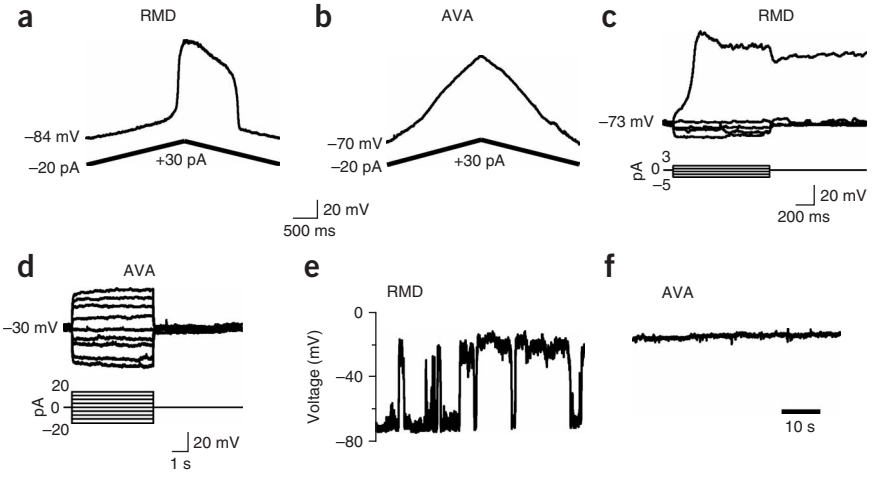

Figure 1 The RMD neuron fires action potentials and shows bistability. (**a**,**b**) Current-clamp records from RMD (**a**) and AVA (**b**) neurons. Voltage (top) in response to a ramp of injected current (bottom) is shown. (**c**,**d**) Voltage responses to small hyperpolarizing and depolarizing current-steps in RMD (**c**) and AVA (**d**) neurons. (**e**,**f**) Voltage when there was zero holding current in RMD (**e**) and AVA (**f**) neurons. Transgenic worms that expressed GFP under the regulation of the *glr-1* promoter (pDM1286) were used for *in vivo* electrophysiological experiments. Electrophysiological recordings from the RMD and AVA neurons *in vivo* were made as described previously[10].

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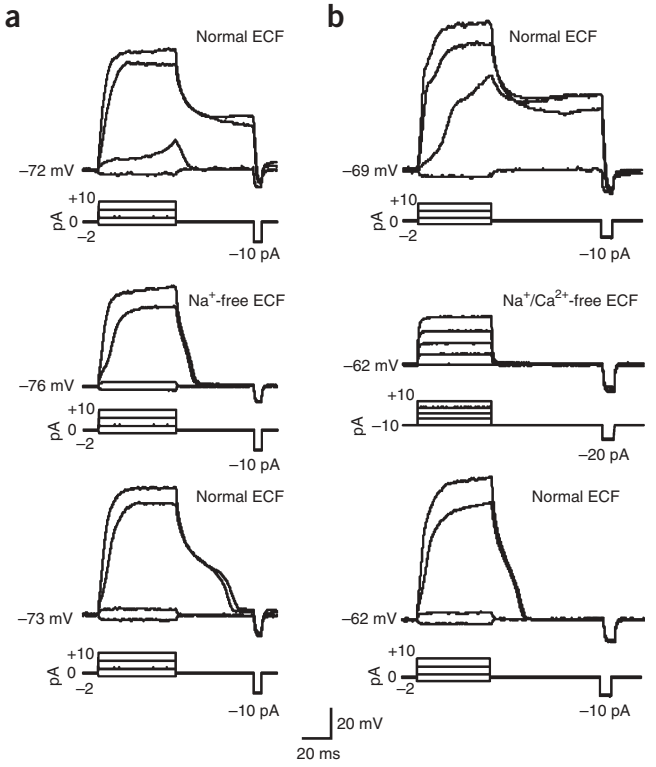

Figure 2 Action potentials in RMD depend on external Ca^{2+}. (**a**,**b**) Action potentials measured in RMD in response to steps of depolarizing current in either normal extracellular fluid (ECF) (top), Na^+-free ECF (middle, **a**) or Na^+/Ca^{2+}-free ECF (middle, **b**), followed by a return to normal ECF (bottom).

contrast, the resting potential of AVA neurons was typically between –20 and –30 mV, and we did not observe action potentials (**Fig. 1d**), even when we changed the resting potential to more hyperpolarized levels (**Supplementary Fig. 1** online). In RMD neurons, we observed long-lived spontaneous fluctuations in membrane potential between the two states, even under conditions in which we injected no current (**Fig. 1e**). Under these same conditions, we did not observe fluctuations between two long-lived states in AVA neurons (**Fig. 1f**), although we did observe fluctuations when the resting potential was changed to more hyperpolarized levels (**Supplementary Fig. 1**). We assume that these fluctuations result from synaptic inputs.

Typically, regenerative changes in potential result from negative slope-conductance regions in the membrane current-voltage relation. Voltage-clamp experiments revealed a substantial, long-lived depolarization-activated inward current in RMD neurons (**Supplementary Fig. 2** online). In contrast, depolarization-activated inward currents were considerably less prominent in AVA neurons, either as a result of intrinsic differences between the neurons or because of differential washout of critical intracellular molecules. Analysis of the current-voltage relations from the voltage-clamp experiments revealed a negative-slope region for RMD, but not for AVA neurons (**Supplementary Fig. 2**). These results raise the possibility that voltage-dependent Ca^{2+} or Na^+ currents underlie the regenerative changes in potential.

Bath application of the Na^+-channel blocker TTX (2 μM) failed to block action potentials (data not shown), which is consistent with genome analysis that has failed to identify conserved voltage-dependent Na^+ channels[2]. In addition, when external Na^+ was replaced by the large cation *N*-methyl-D-glucamine ($NMDG^+$), we still observed action potentials in response to steps of depolarizing current (**Fig. 2a**); however, two changes were apparent. First, for equivalent depolarizing current steps, the onset of action potentials was slightly delayed and their peak amplitude was reduced. Second, the plateau potential following cessation of the current step was eliminated in Na^+-free external solution. Partial recovery from both changes was observed following a return to normal extracellular solution. In contrast, when both external Na^+ and Ca^{2+} were replaced by $NMDG^+$, and the solution was buffered with EGTA, depolarizing current steps no longer elicited action potentials (**Fig. 2b**). These results indicate that Ca^{2+} has a critical role in action-potential generation in RMD.

Vertebrate studies have identified three classes of voltage-gated calcium channels (VGCCs) that differ on the basis of voltage-activation, pharmacology and kinetics. Genetic studies and genome analysis have identified three genes in *C. elegans* (*unc-2*, *egl-19* and *cca-1*) that encode pore-forming alpha subunits of VGCCs and are similar to members of the three classes of vertebrate VGCCs. Thus, *egl-19* encodes an L-type, high voltage–gated channel, *unc-2* encodes a R,N,P/Q–type high voltage–gated channel and *cca-1* encodes a T-type, low voltage–gated channel[11]. In addition, two genes, *nca-1* and *nca-2*, which contribute to sensitivity to anesthetics[12], encode channels homologous to vertebrate NALCN, a voltage-insensitive, nonselective cation channel[13]. To date, no genes have been identified in *C. elegans* that are predicted to encode voltage-gated Na^+ channels[2].

To determine whether these gene products contribute to action potential generation, we recorded voltage responses from wild-type and mutant worms. Compared with wild type (**Fig. 3a**), we observed smaller amplitude and slower onset action potentials in *unc-2*(*e55*) mutants (**Fig. 3b**). Even in this mutant, however, we could observe spontaneous long-lived fluctuations between hyperpolarized and depolarized states (**Fig. 3c**). We found no major changes in action potentials in *egl-19*(*n2368*) and *cca-1*(*ad1650*) single mutants, nor in *nca-2*(*gk5*); *nca-1*(*gk9*) double mutants (**Fig. 3d–f**). These results suggest that multiple classes of Ca^{2+} channels contribute to action potentials, including, perhaps, as yet unidentified channels.

RMD and AVA neurons both contribute to the control of locomotion, express ionotropic glutamate receptors and receive glutamatergic inputs[9]. We have previously described glutamate-gated currents in AVA neurons[13] and we have also found glutamate-gated currents in RMD neurons (data not shown). To test the possibility that sensory input contributes to the apparent bistability of the RMD neurons, we briefly applied 1 mM glutamate to RMD neurons and showed that this was sufficient to trigger an action potential and entry into a long-lived depolarized state ($n = 2$; **Fig. 3g**). In this depolarized state, additional glutamate applications did not appreciably change the potential. RMD neurons could be returned to their original resting potential by a small hyperpolarizing-current injection. Presumably, inhibitory synaptic inputs would switch the neurons back to a more hyperpolarized state. We simulated synaptic currents by injecting current pulses of 10-ms duration into RMD neurons (**Fig. 3h**). We found that a –30-pA current pulse caused the membrane potential to switch back to a hyperpolarized state (range, –5 to –30 pA). Thus, RMD neurons' response to current steps is reminiscent of a Schmitt trigger: the voltage is either low or high depending on whether the input is above or below two separate threshold values. In contrast with what we observed in RMD neurons, glutamate application caused short-lived, modest changes in AVA neuron membrane potential, with no switch to a new steady-state potential ($n = 5$; **Fig. 3i**).

Neurons with processes that extend only short distances (for example, in *C. elegans* or in the outer vertebrate retina) do not necessarily need to communicate via action potentials, as signal degradation may not be substantial over short distances. However,

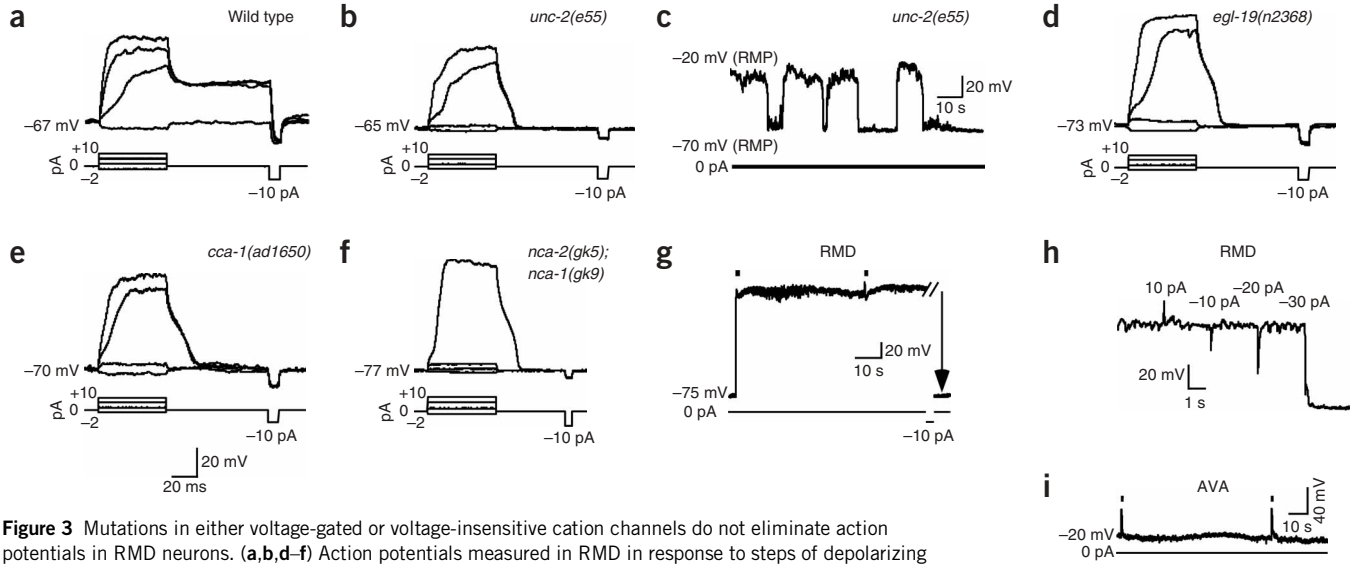

Figure 3 Mutations in either voltage-gated or voltage-insensitive cation channels do not eliminate action potentials in RMD neurons. (**a**,**b**,**d**–**f**) Action potentials measured in RMD in response to steps of depolarizing current in either wild-type (**a**) or mutant worms (**b**,**d**–**f**). (**c**) Bistable hyperpolarized and depolarized resting membrane potentials (RMP) in RMD neurons of *unc-2*(*e55*) mutants held at a current of 0 pA. (**g**) RMD sustained voltage response to brief applications of 1 mM glutamate. Membrane potential switched back to approximately –75 mV (vertical arrow) following a 4-s step of hyperpolarizing current. (**h**) RMD current injections (10 ms), simulating inhibitory synaptic currents, switched the membrane potential to a hyperpolarized state. (**i**) AVA transient voltage response to brief applications of 1 mM glutamate. In **g** and **i**, the short, horizontal bars indicate pressure application of glutamate in a continuous flowing bath.

action potentials might also be used to amplify synaptic signals or contribute to information processing. Rhythmic movements of the worm's nose and the control of forward and backward locomotion are regulated in part by RMD neurons[14], and their Schmitt trigger–like properties could contribute to the switching of these movements and reduce the effects of noise. Neurons that show bistable potentials are also found in vertebrates. For example, cerebellar Purkinje cells, but not granule cells or interneurons, exist in either an up or down state that can be switched by sensory inputs[15], suggesting that bistability is evolutionarily conserved and may contribute to information processing by neural networks.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

J.E.M. carried out the electrophysiological experiments and participated in data analysis. P.J.B. generated strains, participated in data analysis and helped prepare the manuscript. D.M.M. provided molecular biology expertise and A.V.M. supervised the experiments, participated in data analysis and wrote the manuscript.

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1. de Bono, M. & Maricq, A.V. *Annu. Rev. Neurosci.* **28**, 451–501 (2005).
2. Yu, F.H., Yarov-Yarovoy, V., Gutman, G.A. & Catterall, W.A. *Pharmacol. Rev.* **57**, 387–395 (2005).
3. Goodman, M.B., Hall, D.H., Avery, L. & Lockery, S.R. *Neuron* **20**, 763–772 (1998).
4. Davis, R.E. & Stretton, A.O. *Parasitology* **113** Suppl: S97–S117 (1996).
5. Raizen, D.M. & Avery, L. *Neuron* **12**, 483–495 (1994).
6. Shtonda, B. & Avery, L. *J. Exp. Biol.* **208**, 2177–2190 (2005).
7. Davis, R.E. & Stretton, A.O. *J. Neurosci.* **9**, 415–425 (1989).
8. Zheng, Y., Brockie, P.J., Mellem, J.E., Madsen, D.M. & Maricq, A.V. *Neuron* **24**, 347–361 (1999).
9. Brockie, P.J., Madsen, D.M., Zheng, Y., Mellem, J. & Maricq, A.V. *J. Neurosci.* **21**, 1510–1522 (2001).
10. Francis, M.M. & Maricq, A.V. *Methods Mol. Biol.* **351**, 175–192 (2006).
11. Frokjaer-Jensen, C. *et al. J. Neurobiol.* **66**, 1125–1139 (2006).
12. Humphrey, J.A. *et al. Curr. Biol.* **17**, 624–629 (2007).
13. Zheng, Y., Mellem, J.E., Brockie, P.J., Madsen, D.M. & Maricq, A.V. *Nature* **427**, 451–457 (2004).
14. Gray, J.M., Hill, J.J. & Bargmann, C.I. *Proc. Natl. Acad. Sci. USA* **102**, 3184–3191 (2005).
15. Loewenstein, Y. *et al. Nat. Neurosci.* **8**, 202–211 (2005).