Firing Between Two Spike Thresholds: Implications for Oscillating Lobster Interneurons

Abstract. *An identified interneuron in the lobster commissural ganglia fires spikes only between membrane potential values of −60 and −30 millivolts. The membrane potential of this neuron can also oscillate, and interaction between these two properties has important implications in determining the firing pattern of the neuron itself and the modalities of driving of a distant postsynaptic neuron.*

Many neurons function without impulses (*1*), but in some cases, apparently nonspiking neurons can be made to fire spikes by hyperpolarization (*2*), and neurons considered normally spiking can become nonspiking when experimentally depolarized (*3*). Nevertheless, in both these cases, the nonspiking behavior has been considered an artifact resulting from either the experimental procedure or cellular damage. In this report we show that an identified spiking interneuron, the commissural gastric driver (CGD), in the stomatogastric nervous system of the lobster can exhibit spike inactivation as a result of spontaneous depolarization. This phenomenon cannot be attributed to experimental artifact and has important consequences in patterning the activity of distant postsynaptic neurons.

To study the CGD's (*4*), we used an in vitro stomatogastric nervous system preparation (the commissural and stomatogastric ganglia and their connecting and main output nerves) (Fig. 1A) of the lobster *Homarus gammarus* and conventional electrophysiological techniques (*5*). The stomatogastric nervous system is responsible for the generation of cyclical feeding activity, and, specifically, the stomatogastric ganglion contains the central pattern generators for two feeding rhythms: the gastric (trituration) rhythm, which has a long variable period (7 to 16 seconds), and the pyloric (filtration) rhythm, whose period is shorter and less variable (1 to 3 seconds) (*6*). These two pattern generators can function in isolation but they can also be modulated by neurons located in the commissural ganglia. The CGD's are such neurons (*4*).

The CGD cell bodies are located one in each of the two commissural ganglia, which are bilaterally situated on the circumesophageal connectives (Fig. 1A). Although there are no morphological characteristics which single out a CGD from the other neurons in the ganglia, they can be easily identified by their activity and their functional connections. The main criteria for their identification are that each CGD has an axon passing via the ipsilateral superior esophageal nerve and the stomatogastric nerve to the stomatogastric ganglion, where it has an excitatory monosynaptic connection (*4*) with gastric motor neurons (GM's) of the gastric pattern generator (*6*). Furthermore the membrane potential of a CGD can undergo rhythmical oscillations at the same frequency as the gastric motor rhythm, although these oscillations do not depend on the stomatogastric ganglion for their generation (*4*). The behavior and activity to be described can also be considered as diagnostic features of the CGD's.

Intracellular recordings from the cell body of a CGD reveal an unstable oscillating membrane potential which can spontaneously adopt different values during the same experiment (Fig. 1B). As for many arthropod neurons, the cell body is inexcitable and invaded by attenuated electrotonic spikes (middle traces in Fig. 1B). In the CGD, spikes appear

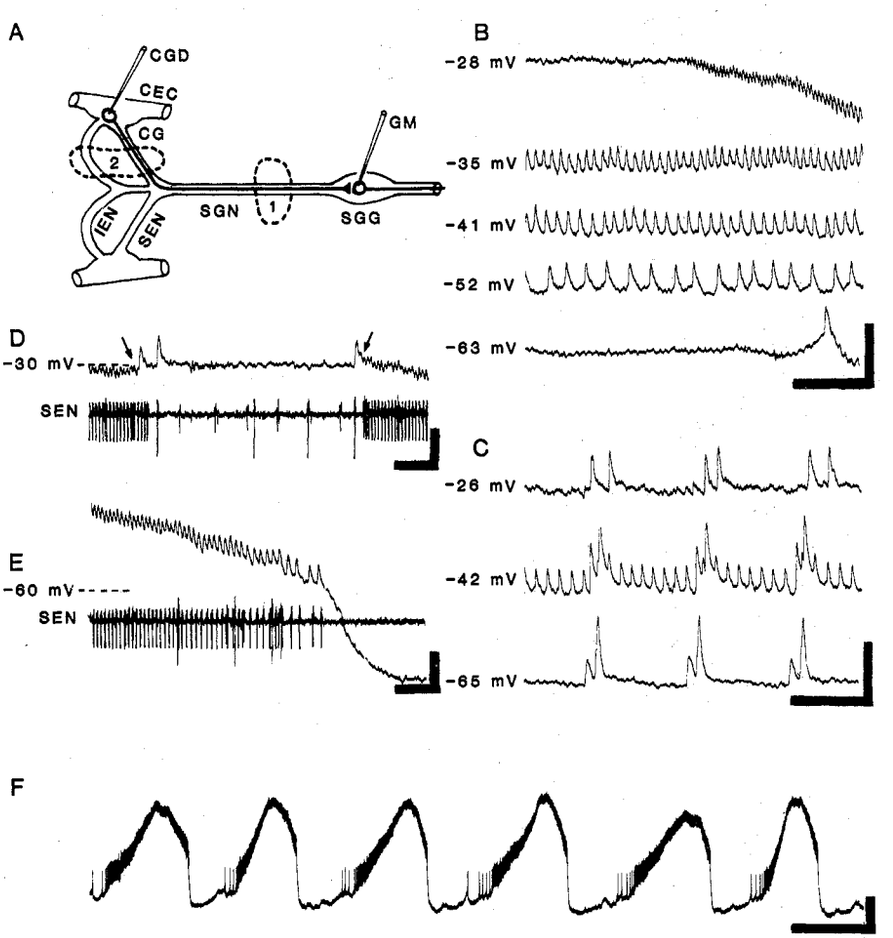

Fig. 1. Inactivation of the spiking discharge of the CGD at low membrane potential as recorded in the cell body (B to E) and on the axon (*SEN* in D and E). (A) The preparation. Abbreviations: *CEC*, circumesophageal connectives; *CG*, commissural ganglion; *IEN*, inferior esophageal nerve; *SEN*, superior esophageal nerve; *SGG*, stomatogastric ganglion; *SGN*, stomatogastric nerve. Vaseline chambers were perfused with an isotonic sucrose solution to isolate the commissural ganglia from the stomatogastric ganglion (1) or to completely isolate a commissural ganglion (2). (B) Soma recording of the spiking activity at different membrane potentials spontaneously adopted by the CGD during a single experiment in which axonal conduction was blocked in the SGN. In the upper trace, the membrane potential crosses the upper threshold (−30 mV) by hyperpolarization and the cell starts spiking. (C) Same as in B but after unblocking of the SGN. The large depolarizing event that is evident when the cell is firing (−42 mV) and when the cell is silent (−26 and −65 mV) is an EPSP generated by an excitatory input coming from the SGG. (D and E) Spike inactivation in the cell body (upper trace) is correlated with spike inactivation in the axon (as recorded extracellularly from the SEN). Spiking stops (D) when the membrane potential crosses the upper threshold (−30 mV) (between arrows) and (E) when the membrane potential crosses the lower threshold (−60 mV). (F) Spontaneous oscillating behavior of the CGD (axonal conduction blocked in the SGN). Scale: horizontal bars, 250 msec except in (F), 10 seconds; vertical bars, 10 mV.

0036-8075/81/1120-0941$01.00/0

only at membrane potentials between −60 and −30 mV. In other words, at low membrane potentials, the spike mechanism is inactivated and spikes reappear (with a very small amplitude and at high frequency) only if the membrane potential crosses an upper threshold of about −30 mV (first trace in Fig. 1B). The recordings of Fig. 1B were obtained in an experiment in which conduction was blocked in the stomatogastric nerve (*5*) (Fig. 1A). When the stomatogastric nerve is not blocked (Fig. 1, C to E, and Fig. 2), recordings taken from the CGD cell body can be complicated by large depolarizing events, the frequency of which is not voltage-dependent. These events are not spikes but excitatory postsynaptic potentials (EPSP's) originating from the stomatogastric ganglion. They appear in the cell body both when the neuron is firing (middle trace in Fig. 1C) and when it is not (first and last traces in Fig. 1C); they are not relevant in the context of this paper.

In the cell body, we recorded only attenuated spikes; such a recording might distort what is really happening at the spike initiating zone. Spike inactivation in the cell body could be due to the failure of spikes to invade the cell body at depolarized membrane potential, that is, at high discharge frequency (*7, 8*). This possibility must be disregarded, however, because when spikes disappear in the cell body (between arrows in trace 1 of Fig. 1D), they also disappear from the axon (trace 2 of Fig. 1D), just as they do when the CGD hyperpolarizes and crosses the lower threshold of −60 mV (Fig. 1E). This simultaneous failure of the spikes, at depolarized membrane potentials, in the cell body and in the axon is also confirmed by the fact that when spiking fails to appear in the cell body, the excitatory effects of CGD on a distant postsynaptic neuron also disappear (see Fig. 2C).

We believe that the depolarized nonspiking state of the CGD cannot be due to cellular damage or experimental procedure for the following reasons. (i) Although it is possible to obtain spike inactivation by depolarization from the spiking state or spike activation by hyperpolarization from the depolarized nonspiking state by current injection into the cell body, these two states can also occur spontaneously in all experiments without any of the experimental conditions being altered. (ii) This behavior can be observed during long-term penetration (sometimes as long as 7 hours). (iii) No other cell penetrated in the same ganglion and under the same experimental conditions in a total of 59 experiments exhibited such a behavior. (iv) The depolarized nonspiking state could be reversibly induced by isolating the commissural ganglion from the rest of the stomatogastric nervous system according to the sucrose gap technique (see Fig. 1A).

The CGD's membrane potential can oscillate (Fig. 1F) and drive the bursting pattern of at least some of the neurons of the gastric pattern generator in the stomatogastric ganglion (*4*). Spike inactivation at low membrane potential would, therefore, have important consequences for the coordination between driver and follower elements within this system. A gastric mill (GM) motor neuron (*6*) receives a strong excitatory input from CGD (Fig. 2, A and B). When the firing frequency of a CGD cell is increased by experimental depolarization (Fig. 2A), the postsynaptic GM neuron is depolarized; when the CGD is experimentally hyperpolarized (Fig. 2B), the EPSP's of the GM disappear, resulting in the overall repolarization of the GM. When the CGD is oscillating spontaneously, if the depolarizing phase of its membrane oscillation is sufficient to cross the upper threshold of −30 mV, spiking stops (Fig. 2C). This results in an abrupt repolarization of the follower GM neuron, which previously was maintained depolarized by the high rate of firing of the CGD (before the arrow in Fig. 2C). Similarly, when the membrane potential of the CGD returns across the upper threshold during the hyperpolarizing phase of its oscillation (Fig. 2D), the cell starts to fire at high frequency and the GM neuron is again depolarized and begins to fire.

Finally the spontaneous oscillating behavior of the CGD (approximately 25 to 30 mV in amplitude) can be observed between different limits of membrane potential. In Fig. 2E, for example, the CGD neuron oscillates between −65 and −35 mV and fires during each depolarized phase of the oscillation. In contrast, the CGD of Fig. 2F oscillates between −50 and −20 mV and consequently fires only during the hyperpolarized phase of

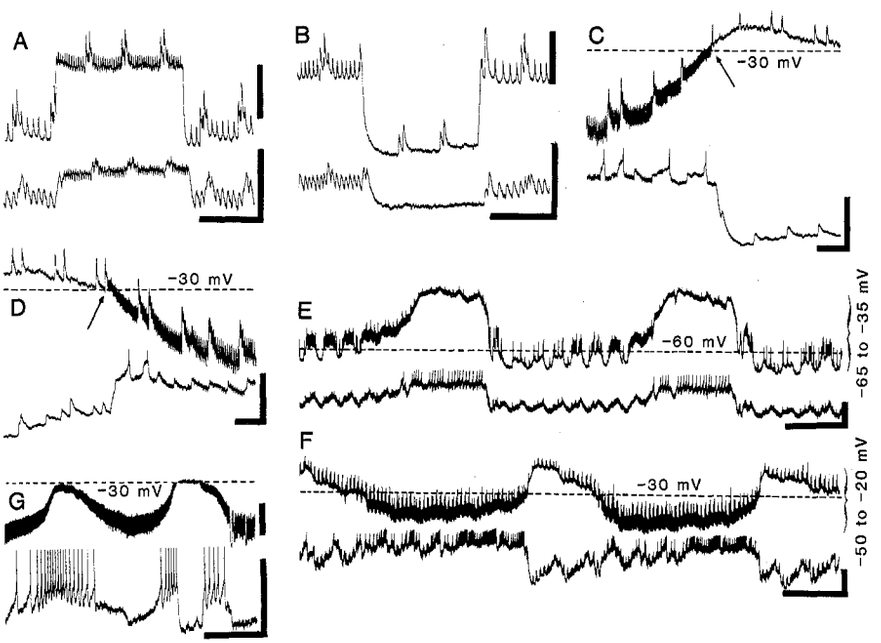

Fig. 2. Driving of a GM postsynaptic neuron in the stomatogastric ganglion (lower traces) by the CGD (upper traces). All the recordings are from the soma of the neurons. (A and B) The GM receives EPSP's correlated 1:1 with the spikes of the CGD. The frequency of these EPSP's is increased when the CGD firing is increased by the injection of depolarizing current (A). This leads to an overall depolarization of the GM while the EPSP's disappear and hyperpolarization of the GM when CGD firing is stopped by experimental hyperpolarization (B). In (B) the large EPSP that appears in the CGD during hyperpolarization does not affect the GM membrane potential. (C) Spontaneous depolarization of the CGD from a spiking state to a nonspiking state (arrow) stops its excitatory effect on the GM, which hyperpolarizes immediately. (D) Conversely spontaneous hyperpolarization of the CGD from a nonspiking to a spiking state (arrow) restarts the excitatory effects on the GM. (E and F) When the CGD membrane potential oscillates, the GM can be driven to burst in phase (E) or out of phase (F) with the oscillation. In (E) the CGD oscillates between −65 and −35 mV and fires by depolarization; in (F) it oscillates between −50 and −20 mV and fires by hyperpolarization. Larger events on CGD recordings are EPSP's. (G) When the membrane potential of CGD crosses the upper inactivation threshold (−30 mV) it interrupts the corresponding GM cycle, producing two GM bursts for each CGD cycle. Scale: horizontal bars, 250 msec except in (E, F, and G), 5 seconds; vertical bars, 10 mV.

each oscillation. Such variation in the intrinsic behavior of CGD neurons causes a corresponding alteration in the phasing of the driven responses in the postsynaptic GM cells. Whereas the GM neuron of Fig. 2E is depolarized (and fires its burst of spikes) during the depolarized phase of the presynaptic CGD, in the second case (Fig. 2F) it is driven depolarized by the hyperpolarized phase of the CGD. Thus a shift in the mean membrane potential about which a CGD oscillates seems able to switch the phase relationships between the oscillations of presynaptic and postsynaptic neurons. This shift could be mediated by tonic (excitatory or inhibitory) inputs to the CGD. That such tonic inputs exist is indicated by the fact that complete isolation of the commissural ganglion (Fig. 1A) can reversibly induce the depolarized nonspiking state in CGD, presumably by the removal of some inhibitory influence.

The CGD function thus seems to depend on an interaction between the cell's two membrane potential thresholds and the absolute limits of membrane potential oscillation. An alteration in oscillation (by tonic or phasic influences) results in dramatic postsynaptic effects, one of which is to change the phase relationships between the activity of the CGD and its follower GM neuron. A further effect is that a small change in the depolarized maximum of an oscillatory CGD cell can cause spike inactivation during the peak of oscillation and result in an immediate doubling of GM burst frequency (Fig. 2G). That is, the coupling between driver and follower has changed from 1:1 to 1:2 cycle coordination.

Our results demonstrate that a spiking neuron can become nonspiking at both high and low levels of membrane potential. For an oscillatory neuron, which is subject to control by tonic influences, this provides a means of generating different patterns of rhythmic output. In the context of behavioral flexibility, therefore, the cellular mechanism described here could play a major functional role. It remains to be seen whether this mechanism is a widespread phenomenon among rhythm pattern generators.

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References and Notes

1. A. Roberts and B. M. H. Bush, Eds., *Neurons Without Impulses* (Cambridge Univ. Press, Cambridge, 1981).
2. R. Hengstenberg, *Nature (London)* **270**, 338 (1977).
3. F. Werblin, *J. Physiol. (London)* **264**, 767 (1977); M. Murakami and Y. Shimoda, *ibid.*, p. 801.
4. R. M. Robertson and M. Moulins, *Neurosci. Lett.* **21**, 111 (1981).
5. The nervous system was dissected from the excised stomach of a lobster in saline (artificial seawater) and pinned in a Sylgard-lined petri dish. The preparation was continuously perfused with cool, oxygenated saline. Vaseline chambers were formed around the stomatogastric nerve and around the superior and inferior esophageal nerves (1 and 2 in Fig. 1A). These could then be perfused separately with an isotonic (740 m*M*) sucrose solution for conduction blockade. Extracellular recordings were made with fine platinum wire electrodes and intracellular recordings from the cell bodies with glass microelectrodes filled with 3*M* KCl and pulled to a resistance of 15 to 30 megohms. Neurons in the stomatogastric ganglion were identified according to the muscles they innervated.
6. D. M. Maynard, *Ann. N.Y. Acad. Sci.* **193**, 59 (1972); A. I. Selverston, D. F. Russell, J. P. Miller, D. G. King, *Prog. Neurobiol.* **7**, 215 (1976).
7. M. R. Spira, Y. Yarom, I. Parnas, *J. Neurophysiol.* **39**, 882 (1976); D. O. Smith, *J. Physiol. (London)* **301**, 243 (1980).
8. Y. Grossman, I. Parnas, M. E. Spira, *ibid.* **295**, 307 (1979).
9. Supported by grant 80 P 6049 from the Delegation Generale à la Recherche Scientifique et Technique and by a European Science Exchange Fellowship to R.M.R. by the Royal Society and the Centre National de la Recherche Scientifique. We thank F. Delcomyn, F. Nagy, K. G. Pearson, A. Selverston, and J. Simmers for critically reviewing successive drafts of the manuscript.

4 May 1981; revised 15 July 1981