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PLATEAU POTENTIALS IN MOTOR NEURONS IN THE VENTILATORY SYSTEM OF THE CRAB

RALPH A. DiCAPRIO*

Neurobiology Program, Ohio University, Department of Biological Sciences, Irvine Hall, Athens, OH 45701, USA

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Summary

The motor neurons in the crab ventilatory system have previously been considered to be passive output elements in that the generation of bursts of action potentials in these neurons during ventilation was thought to be due to cyclic inhibition and excitation from the interneurons in the ventilatory central pattern generator. This study demonstrates that the large-amplitude depolarization that underlies bursts of action potentials in ventilatory motor neurons is produced by a plateau potential. These motor neurons satisfy a number of the experimental tests that have been proposed for plateau potentials, such as triggering of the burst by a brief depolarization, termination of the burst by a hyperpolarizing input, and an all-or-none suppression of the depolarizing potential by the injection of hyperpolarizing current.

Key words: shore crab, *Carcinus maenas*, ventilation, central pattern generator, plateau potential.

Introduction

The motor pattern that a central pattern generator (CPG) produces is determined by the synaptic interactions among, and intrinsic membrane properties of, the neurons that make up the CPG network. In early studies of rhythmic motor systems, motor neurons were usually considered to be 'passive' output elements whose firing properties were strictly dependent upon the pattern of synaptic inputs integrated by these neurons. In recent years, it has become apparent in a variety of invertebrate and vertebrate motor systems that motor neurons may possess a wide range of intrinsic voltage-dependent properties that actively transform their response to driving inputs. One such intrinsic property results in the generation of plateau potentials (Russell and Hartline, 1978; for reviews, see Kiehn, 1991; Marder, 1991). Plateau properties transform a motor neuron from a simple summing junction for external inputs into a quasi-bistable neuron, in which a depolarization of sufficient amplitude triggers a regenerative, sustained, depolarizing current that drives the neuron to a depolarized voltage that may be superthreshold for the generation of action potentials. These triggering inputs may be caused by excitatory synaptic inputs or result from post-inhibitory rebound after a period of hyperpolarization due to the presence of a hyperpolarization-activated inward current, I_h (Arbas and Calabrese, 1987; Hounsgaard and Kiehn, 1989). Plateaus can be terminated by intrinsic repolarizing (outward) currents in the motor neuron that are activated by the plateau-mediated depolarization or by hyperpolarizing synaptic inputs to the neuron. Plateau potentials have been found in the motor neurons of both invertebrate and vertebrate motor systems, such as the stomatogastric system (Russell and Hartline, 1978), and in the crayfish *Pacifastacus leniusculus* (Sillar and Elson, 1986), the cockroach *Periplaneta americana* (Hancox and Pitman, 1991), the locust *Locusta migratoria* (Rameriz and Pearson, 1991), the lamprey (Wallén and Grillner, 1987), the cat (Hounsgaard *et al.* 1984, 1988) and the turtle *Pseudemys scripta* (Hounsgaard and Kiehn, 1989; Hounsgaard and Kjærulff, 1992).

The present study demonstrates that plateau properties are a major feature underlying the bursting of motor neurons in the crab ventilatory motor system.

Materials and methods

Preparation

Male and female green shore crabs *Carcinus maenas* (L.) were used in all experiments. The animals were maintained for 2–10 weeks in artificial seawater aquaria at 10 °C until use. The isolated ganglion preparation used in this study has been described in detail elsewhere (Simmers and Bush, 1983*a*; DiCaprio and Fourtner, 1984). Briefly, the walking legs and chelae were autotomized, and the dorsal carapace, viscera and brain were removed. The thorax was pinned down in a Sylgard-lined dish containing oxygenated crab saline (Ripley *et al.* 1968). The sternal artery was immediately cannulated and the thoracic ganglion perfused with oxygenated saline at a rate of 2–3 ml min^{-1}. The nerves to both scaphognathites (SGs) were dissected from the SG musculature and all remaining nerves from the thoracic ganglion were severed. The thoracic ganglion was removed from the thorax and pinned dorsal surface up on

*e-mail: rdicaprio1@ohiou.edu.

an inclined Sylgard platform. This exposed the dorsolateral aspect of the thoracic ganglion, which was desheathed with fine forceps to permit intracellular recording from the underlying ventilatory neuropil. All experiments were performed at room temperature (21–22 °C). The isolated ganglion preparation used in this study spontaneously expresses the motor pattern corresponding to forward ventilation for periods of 6–8 h, with a typical intrinsic frequency of 0.65–1.0 Hz. Pauses in the ventilatory motor output and spontaneous bouts of reverse ventilation occurred infrequently in all experiments.

Recording procedures

Extracellular recordings of the ventilatory motor pattern were made with polyethylene suction electrodes placed on the cut ends of the SG levator (LEV) and depressor (DEP) motor nerves from either the left- or right-hand side of the ganglion. In most experiments, recordings from the levator nerve were made proximal to the branch of this nerve that innervates depressor muscle D2a, thereby allowing recording of the D2a motor neuron along with levator motor neuron activity. A suction electrode was also placed on the intact circumesophageal connective ipsilateral to the site of intracellular recording to permit stimulation of descending fibers that start, stop or alter the rate of the ventilatory rhythm (Wilkens *et al.* 1974). Connective stimulation was made with continuous pulse trains (200 μs duration pulses) at frequencies of 40–100 Hz. Intracellular recordings were obtained from neuropilar processes of ventilatory motor neurons with glass microelectrodes filled with 2 mol l^{-1} potassium acetate with resistances in the range 25–30 MΩ. Intracellular voltages were amplified using a bridge electrometer (WPI 767). All signals were recorded on an eight-channel instrumentation tape recorder (HP model 3968A) for later analysis and reproduced on an AstroMed model MT8800 chart recorder.

Ventilatory motor neurons were identified on the basis of their rhythmic firing in phase with the extracellularly recorded motor pattern, by (intracellular) spike-triggered averaging of the extracellular recording from the appropriate motor nerve and by antidromic activation of the motor neuron by extracellular stimulation of the motor nerve.

Results

Gill ventilation in decapod Crustacea is produced by the rhythmic dorso-ventral movements of the scaphognathite (SG) or gill bailer of the second maxilla. The beating of the SG pumps water through the branchial chamber and over the gills. Movement of the SG is controlled by five depressor muscles, which have been classified into two functional groups, Dep1 and Dep2, and five levator muscles, also divided into two groups, Lev1 and Lev2 (Young, 1975). Movement of the SG can pump water in either of two directions, corresponding to forward and reverse ventilation. In the forward mode of ventilation, water is drawn through the branchial chamber, entering at the base of the chelae (the Milne-Edwards openings) and at analogous openings at the base of the walking legs. Water passes through the branchial chambers and over the gills before exiting *via* anterior exhalant channels located under the antennae. Forward pumping is the prevalent mode in *Carcinus maenas* (Wilkens, 1976) and is characterized by the muscle recruitment sequence Dep1–Dep2–Lev1–Lev2. Reverse ventilation, where water enters anteriorly and exits at the base of the legs, results from a change in the recruitment sequence of the SG muscles to Dep2–Dep1–Lev2–Lev1. The motor neurons which drive the Dep2 and Lev2 muscle groups during forward ventilation become silent during reverse ventilation; during reverse ventilation, these muscle groups are driven by a distinct set of ‘reversal’ motor neurons that only spike when the reverse motor pattern is expressed (Young, 1975; Simmers and Bush, 1983*b*).

The motor patterns controlling the left and right SGs are produced by separate CPGs (Mendelson, 1971; Wilkens and Young, 1975). The CPG responsible for the production of ventilatory pumping in Crustacea was originally thought to consist of a single endogenously oscillating nonspiking neuron (Mendelson, 1971), but was subsequently shown to consist of at least two nonspiking neurons (Simmers and Bush, 1983*a*) and is now known to incorporate eight nonspiking interneurons (DiCaprio, 1989), three frequency-modulating interneurons (DiCaprio and Fourtner, 1988) and a single interneuron that mediates the switch from forward to reverse ventilation (DiCaprio, 1990). The motor neurons in the ventilatory system of the crab have previously been considered to be passive output elements driven primarily by a cyclic inhibition (Simmers and Bush, 1983*b*) from the ventilatory CPG, or possibly by a combination of tonic excitatory and cyclic inhibitory inputs (DiCaprio and Fourtner, 1984). Recordings were obtained from 35 motor neurons (29 forward and six reversal motor neurons), with a minimum of five recordings from neurons in each of the four motor groups.

A typical intracellular recording from a ventilatory motor neuron during forward ventilation is shown in Fig. 1. This Dep1 group motor neuron is representative of all of the motor neurons in the ventilatory system in that it fires in bursts elicited by large-amplitude depolarizations of 15–30 mV peak-to-peak depolarization. The maximum hyperpolarized level of this neuron was −65 mV, and the resting membrane potential during pauses in the ventilatory rhythm was −59 mV, which was below the threshold for the production of action potentials. Note that the amplitude of the intracellularly recorded action potentials is approximately 6 mV, reflecting the electrotonic distance of the recording electrode in the neuropil from the spike-initiating region, which is located near the point where the axon exits the thoracic ganglion (Simmers and Bush, 1983*a*). The hyperpolarized portion of the membrane potential oscillation results from a synaptic inhibition that is accompanied by a large increase in membrane conductance and is therefore presumably chemically mediated (Simmers and Bush, 1983*b*; DiCaprio and Fourtner, 1984). Previous studies have suggested that the depolarizing phase of the oscillation was produced either by

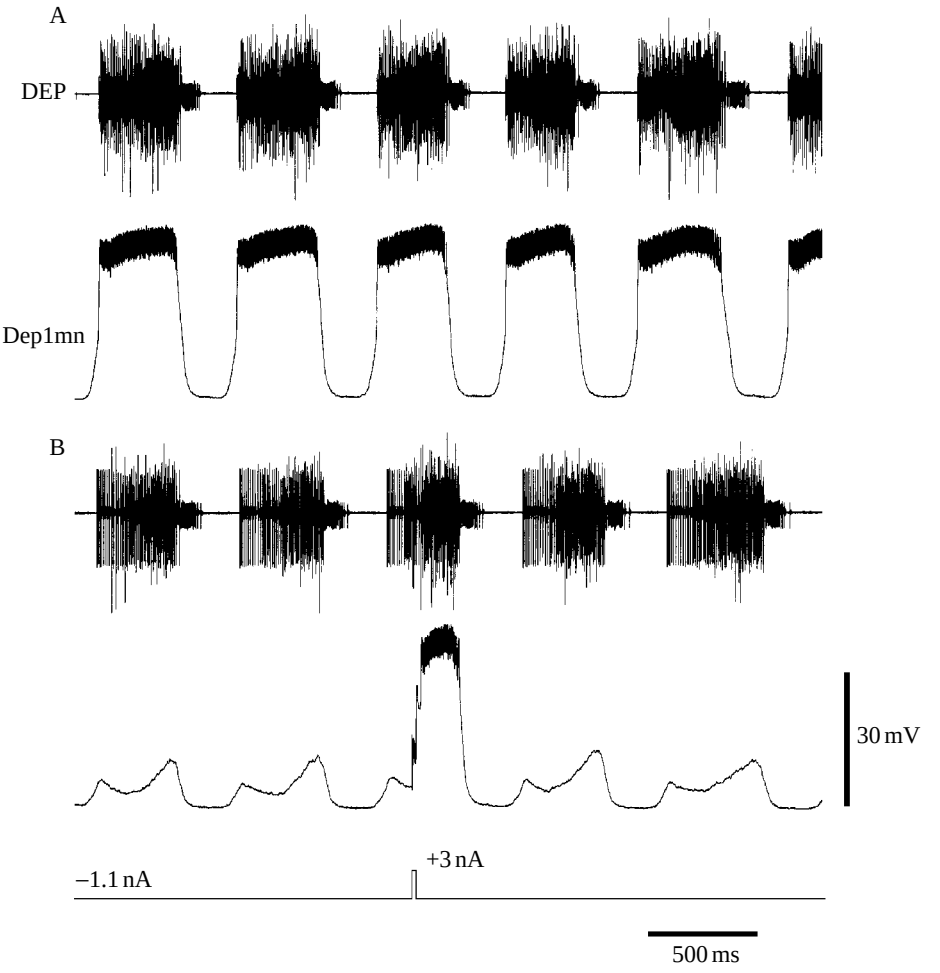

Fig. 1. The motor neurons in the crab ventilatory system are driven by large-amplitude membrane potential oscillations. (A) An intracellular recording from a depressor Dep1 group motor neuron (Dep1mn) during forward ventilation. The maximum peak-to-peak amplitude of the membrane potential (lower trace) underlying the motor burst (upper trace) is approximately 30 mV and the maximum hyperpolarized potential during the oscillation is −65 mV. (B) Injection of a hyperpolarizing current of 1.1 nA into the same motor neuron causes an abrupt all-or-none decrease in the amplitude of the membrane potential oscillation to a maximum peak value of 9 mV. The injection of a 20 ms current pulse (bottom trace) in addition to the sustained hyperpolarizing current (net amplitude +3 nA) triggers a plateau potential in the motor neuron. This plateau potential and the resultant burst of action potentials are terminated at the normal phase in the ventilatory motor pattern by a cyclic synaptic inhibition from the ventilatory central pattern generator. DEP, extracellularly recorded ventilatory motor pattern.

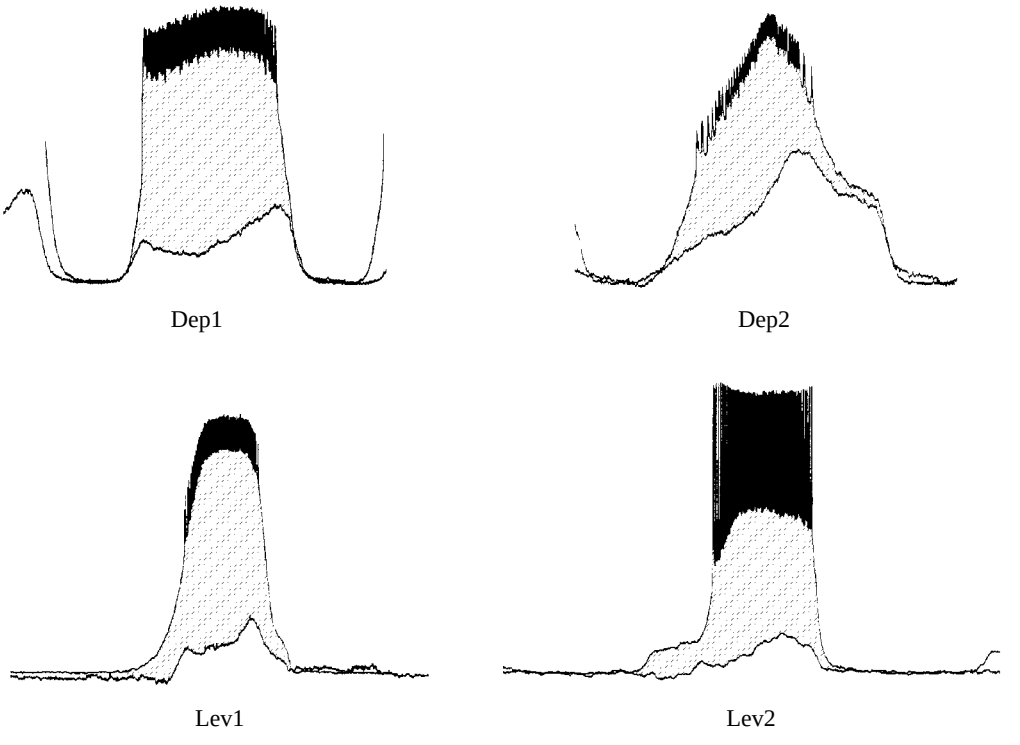

Fig. 2. Plateau potentials in ventilatory motor neurons in the four different groups determined in the same manner as Fig. 1. Each motor neuron showed motor bursts during forward ventilation, and the large-amplitude oscillations in their membrane potentials were suppressed by the injection of sustained hyperpolarizing current in the range −0.7 to −1.3 nA. In each part of the figure, the normal burst and the membrane potential of the neuron during the hyperpolarization are super-imposed. The records were aligned using the extracellularly recorded motor pattern as a reference (not shown). The Dep1 record is for the neuron shown in Fig. 1. The area between the curves is shaded to emphasize the difference in the membrane potential oscillation between the normal and hyperpolarized recordings. Lev1,2, levator group 1 or 2 motor neuron.

removal of inhibition and a post-inhibitory rebound depolarization (Simmers and Bush, 1983*b*) or by a tonic excitation in conjunction with the cyclic inhibitory input (DiCaprio and Fourtner, 1984).

Fig. 1B is a recording from the same motor neuron during the intracellular injection of a constant hyperpolarizing current. The amplitude of the membrane potential oscillation is markedly decreased and this decrease occurs in an all-or-none manner with the injection of approximately −1.1 nA. If a brief (net) depolarizing current pulse (+3 nA, 20 ms) is injected during the sustained hyperpolarization, the large-amplitude depolarizing phase of the oscillation is restored for the normal duration of the neuron's activity in the motor pattern. In this particular experiment, the minimum magnitude of the depolarizing current pulse required to elicit a depolarization was approximately +2 nA. This current pulse elicits this response if it is injected during the time when the neuron would normally be depolarized. The motor burst is terminated at the usual point in the motor pattern owing to the inhibitory input from the ventilatory CPG. Injections of larger-amplitude and longer-duration depolarizing pulses during the normal hyperpolarized (inhibitory) phase were ineffective in restoring the burst, although the neuron could be driven sufficiently to produce action potentials for the duration of a depolarizing current pulse. It would therefore appear that the large depolarizations underlying the bursting of this motor neuron are produced by a plateau potential.

Similar evidence for plateau potentials in other ventilatory motor neurons were obtained for all of the neurons tested (N=35) in each of the four motor groups that constitute the ventilatory motor pattern. In all cases, injection of constant hyperpolarizing current of between −0.7 and −1.3 nA into

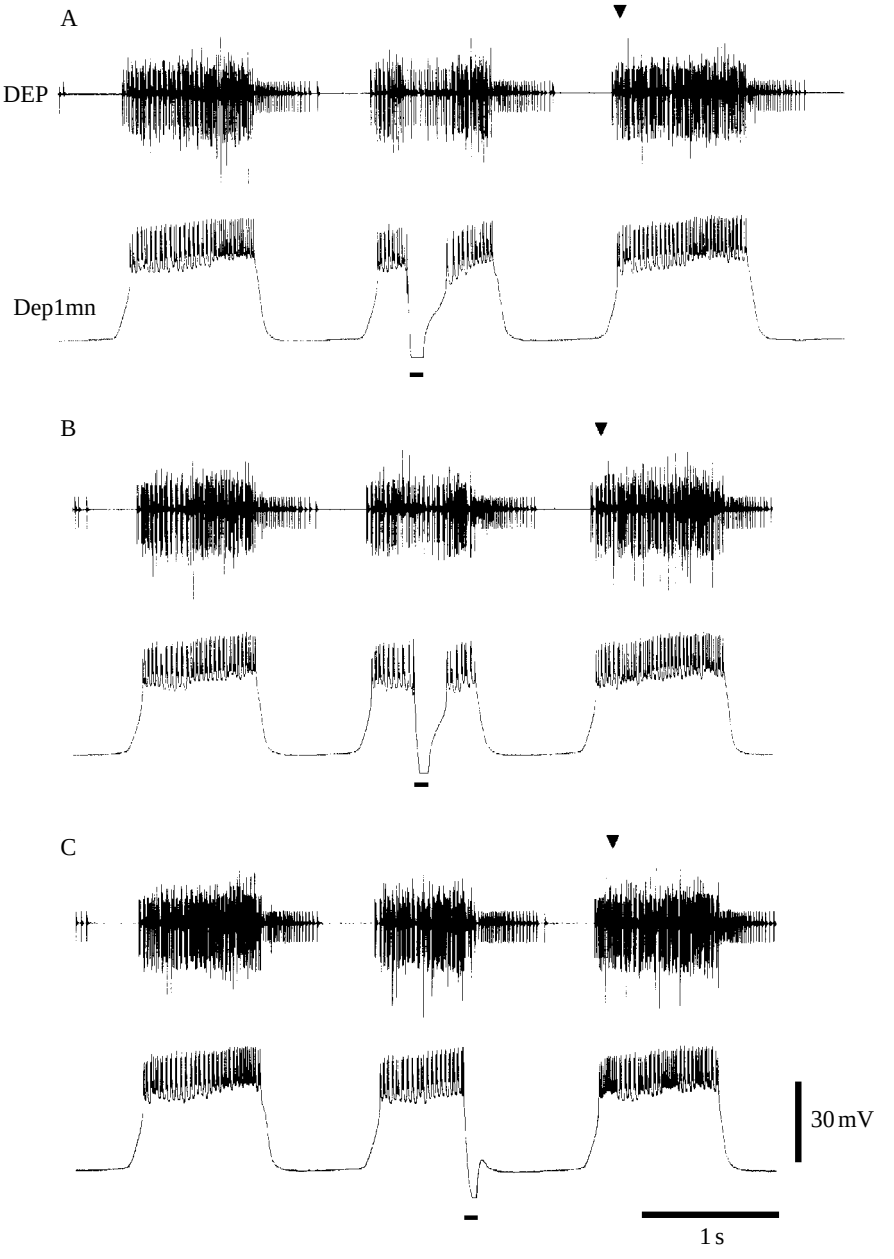

Fig. 3. (A,B) Two examples demonstrating that injection of brief hyperpolarizing current (bar; 100 ms, −4 nA,) pulses into a depressor Dep1 group motor neuron (Dep1mn) did not terminate the plateau potential in the motor neuron. At the end of the current pulse, the motor neuron depolarizes and returns to the same membrane potential as during the initial portion of the burst. Although injection of the current pulse could decrease the duration of the depressor burst in the cycle if the pulse was applied near the end of the burst (C), this is probably due to the resetting of the ventilatory rhythm. The expected start of the next depressor burst following the cycle where the pulse was injected is indicated by the arrowhead above each depressor record (DEP).

ventilatory motor neurons caused an abrupt all-or-none decrease in the amplitude of the membrane potential oscillation, and the small-amplitude membrane potential oscillations that remained were subthreshold for the production of action potentials. Fig. 2 demonstrates the existence of plateau potentials in four of these neurons. In each case, an intracellular recording of a normal motor burst has been superimposed on a recording obtained while the neuron was held hyperpolarized by current injection. The shaded area indicates the difference between the membrane potential oscillation observed before and during the current injection. In all cases, the injection of a brief net depolarizing current pulse during the period when the neuron would normally burst could restore the burst, which was then terminated by the cyclic inhibitory input to the neuron.

Given that plateau potentials may be terminated by inhibitory input (Russell and Hartline, 1978), it might be expected that injection of a brief hyperpolarizing current pulse into ventilatory motor neurons would end the plateau. However, when the ventilatory CPG is active, hyperpolarizing pulses could not terminate a motor neuron burst unless the pulse was injected close to the end of the normal burst. For example, in the depressor Dep1 group motor neuron shown in Fig. 3, hyperpolarizing pulses injected early in a burst interrupt the firing of the neuron (Fig. 3A,B), but there is a return to the depolarized plateau level at the end of the pulse. The accelerating trajectory of the membrane potential during depolarization of the neuron is characteristic of the regenerative increase in membrane potential expected during the initiation of a plateau potential (Russell and Hartline, 1982). The inability of the hyperpolarizing pulse to terminate the plateau potential indicates that the neuron remains sufficiently depolarized at the end of the pulse to permit the retriggering of the plateau potential in the absence of additional imposed hyperpolarization. This may be due to a tonic excitatory input or to the presence of a hyperpolarization-activated inward current that is activated by the hyperpolarizing pulse (see Fig. 9). In all cases, the burst duration is altered when the current pulse is applied and the motor pattern is reset (DiCaprio and Fourtner, 1984). In this case, the current pulse caused a phase advance in the motor pattern; that is, the depressor burst in the cycle immediately after the pulse was applied occurred earlier than would be predicted from the timing of the preceding cycle period. Note that the plateau is terminated prematurely when the current pulse was applied late in the burst (Fig. 3C), but that the duration of the entire Dep1 burst is decreased owing to the resetting of the motor pattern. The termination of the plateau is therefore probably due to the phase shift in the ventilatory CPG, and the consequent earlier inhibition from the CPG, instead of being independently terminated by the current injection into the motor neuron.

However, if a motor neuron was hyperpolarized with d.c. injection so that the threshold for plateau generation was not reached, a plateau potential that was triggered by a depolarizing pulse could be terminated by the injection of a brief hyperpolarizing pulse. In Fig. 4A, a Dep1 group motor neuron was held below the threshold for the initiation of a plateau potential by the injection of −1.2 nA of intracellular current. A brief net depolarizing current pulse (+2.5 nA) injected at the beginning of the small depolarizing oscillation elicits a plateau potential and burst of action potentials and, as seen before, the burst is then terminated by inhibition from the CPG. Under the same conditions, a current-induced plateau could be terminated before the inhibition from the CPG by the injection of a brief hyperpolarizing current pulse (Fig. 4B). The premature termination of the large-amplitude depolarization by a hyperpolarizing current pulse satisfies

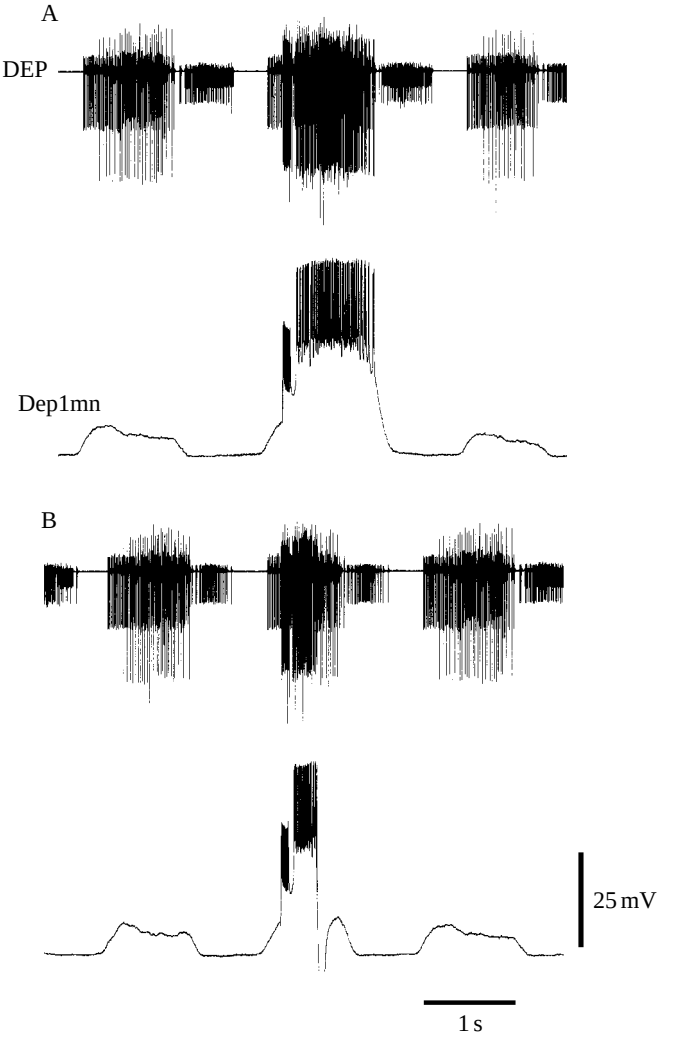

Fig. 4. Plateau potentials in ventilatory motor neurons can be terminated by hyperpolarizing current pulses if the burst was initiated by a depolarizing pulse during a sustained hyperpolarization. (A) Intracellular recording from a depressor Dep1 group motor neuron (Dep1mn) when plateau potentials were suppressed by a constant 1.2 nA hyperpolarizing current. Injection of a net +2.5 nA, 80 ms current pulse triggers a plateau potential in the motor neuron which is then terminated by the inhibition from the ventilatory central pattern generator. (B) A depolarizing current pulse was again used to elicit a plateau potential, which was then terminated prematurely by the application of a (net) −3.7 nA current pulse. DEP, extracellularly recorded ventilatory motor pattern.

another of the criteria proposed for the determination of plateau potentials (Russell and Hartline, 1982).

Plateau potentials in reversal motor neurons

The ventilatory CPG can produce two distinct motor patterns corresponding to forward and reverse ventilation. The data presented above were obtained from motor neurons that are active during forward ventilation. During reverse ventilation, most of these 'forward' motor neurons do not generate bursts of action potentials, but instead only receive a cyclic inhibition (Simmers and Bush, 1983*a*; DiCaprio and Fourtner, 1984). During reverse ventilation, the Dep2 and Lev2 group muscles are instead driven by a set of 'reversal' motor neurons (Young, 1975; Simmers and Bush, 1983*a*; DiCaprio and Fourtner, 1984). During forward ventilation, the membrane potentials of the reversal motor neurons oscillate in phase with the (forward) ventilatory pattern owing to a cyclic inhibitory input (Simmers and Bush, 1983*a*; DiCaprio and Fourtner, 1984), but this oscillation is subthreshold for the initiation of action potentials. However, if these cells are tonically depolarized during forward ventilation by the injection of intracellular current, they will fire bursts of action potentials in phase with the forward ventilation motor pattern (Simmers and Bush, 1983*a*). Previous studies had suggested that the reversal motor neurons receive a tonic excitatory drive during reverse ventilation which, when summed with the cyclic inhibition, results in the production of bursts of action potentials (Simmers and Bush, 1983*a*; DiCaprio and Fourtner, 1984).

Injection of brief depolarizing current pulses into reversal motor neurons during forward ventilation triggers a plateau potential and a burst of action potentials. Fig. 5 shows an intracellular recording from a levator (Lev2 group) reversal motor neuron during forward ventilation. There is a small-amplitude (approximately 8 mV) membrane potential oscillation in phase with the forward motor pattern that is produced by a cyclic inhibitory input. A brief depolarizing current pulse elicits a plateau potential in this cell, which was terminated by the inhibitory drive to this neuron (Fig. 5A). Note that this current pulse also resets the (forward) ventilatory motor pattern. In other trials, the plateau potential was sometimes followed by a few cycles of the reverse ventilation motor pattern (Fig. 5B), in which the bursts in the reversal motor neuron are very similar to the initial burst triggered by the depolarizing pulse. This indicates that reversal motor neurons can generate plateau potentials during forward ventilation and that the bursts of reversal motor neurons are probably dependent upon plateau properties during the expression of the reverse ventilatory motor pattern.

Plateau potentials could be terminated in reversal motor neurons by applying a brief hyperpolarizing current pulse during the plateau. Fig. 6 is a intracellular recording from a depressor Dep2 group reversal motor neuron during forward ventilation. The maximum hyperpolarized membrane potential of this neuron was −69 mV and the peak-to-peak amplitude of the membrane potential oscillation is approximately 6 mV. When an 80 ms depolarizing current

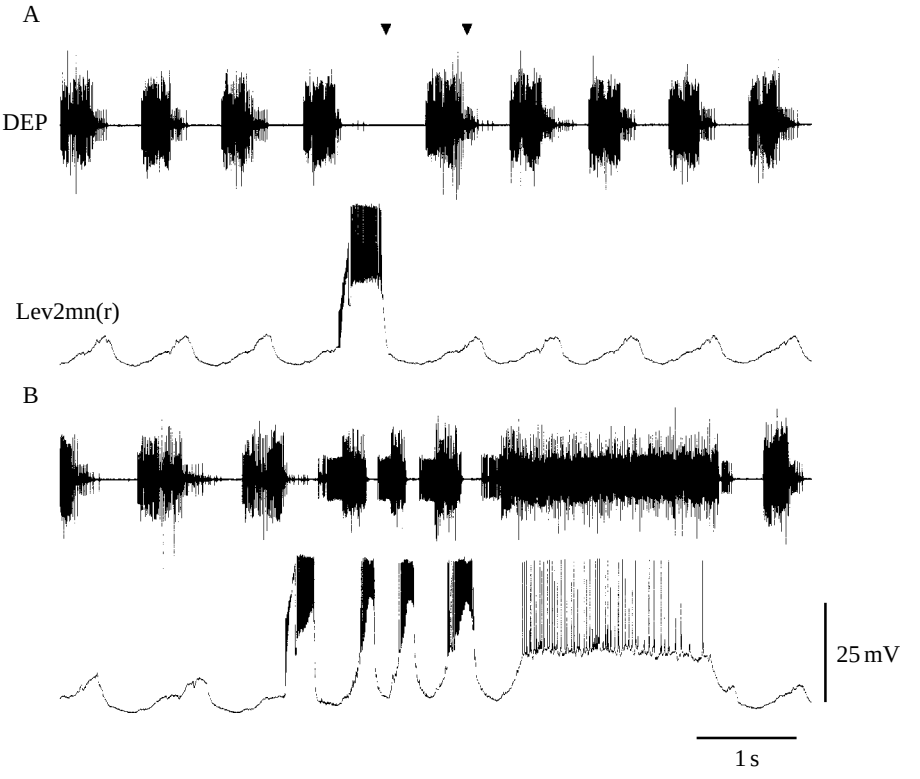

Fig. 5. Plateau potentials in reversal motor neurons can be triggered by depolarizing current pulses injected during forward ventilation. (A) An intracellular recording from a levator group 2 reversal motor neuron, Lev2mn(r), during forward ventilation. Reversal motor neurons receive a cyclic inhibitory input in phase with the forward ventilatory rhythm. If a brief (100 ms) depolarizing current is injected into this motor neuron during the depolarized phase of its oscillation, a large-amplitude plateau potential is triggered in the neuron. Note that the current pulse also resets the forward ventilatory rhythm. The expected start of the first two depressor (DEP) bursts after the injection of the current pulse is indicated by the arrowheads. (B) In some trials, the current pulse triggered a plateau potential which was followed by 3–5 cycles of the reverse ventilation motor pattern. Injection of depolarizing current into reversal motor neurons usually initiated reversed ventilation due to the electrical coupling between reversal motor neurons and the reversal switch interneuron (R. A. DiCaprio, unpublished observations). Note the similarity between the amplitude of the membrane potential oscillation during reverse ventilation and the burst that was initially triggered by the depolarizing current pulse.

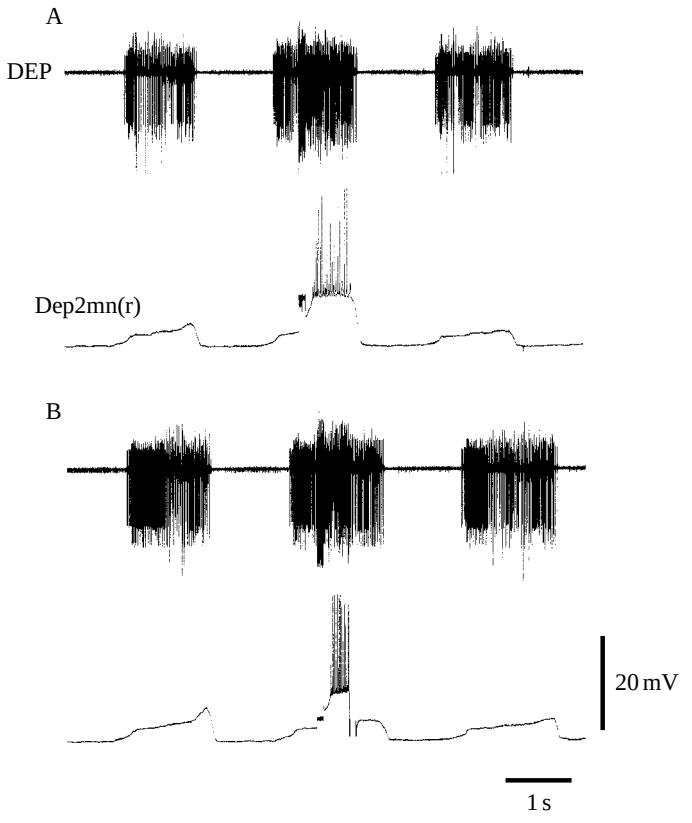

Fig. 6. Plateau potentials in reversal motor neurons can be terminated by the intracellular injection of a hyperpolarizing current pulse. A depolarizing current pulse (100 ms) injected into a depressor reversal motor neuron, Dep2mn(r), during forward ventilation (A) will elicit a plateau potential, which is terminated by the inhibitory input from the central pattern generator (CPG). A current-triggered plateau potential can be terminated (B) by the injection of a hyperpolarizing pulse prior to the CPG-derived inhibition. DEP, extracellularly recorded ventilatory motor pattern.

pulse (+2.0 nA) was injected into the motor neuron, a plateau potential was elicited that was then terminated by the cyclic inhibitory input that the neuron receives during forward ventilation (Fig. 6A). A current-induced plateau could also be terminated before the 'normal' end of the burst by a brief hyperpolarizing current pulse (Fig. 6B). Similar results were obtained for all of the reversal motor neurons (*N*=6) encountered in this study.

Motor neuron input resistance during the ventilatory cycle

The production of plateau potentials as the basis for ventilatory motor neuron bursts is further supported by examination of the change in input resistance of these motor neurons during the ventilatory cycle. Small-amplitude (−0.5 nA) hyperpolarizing current pulses (80 ms duration) were injected into a depressor Dep1 group motor neuron

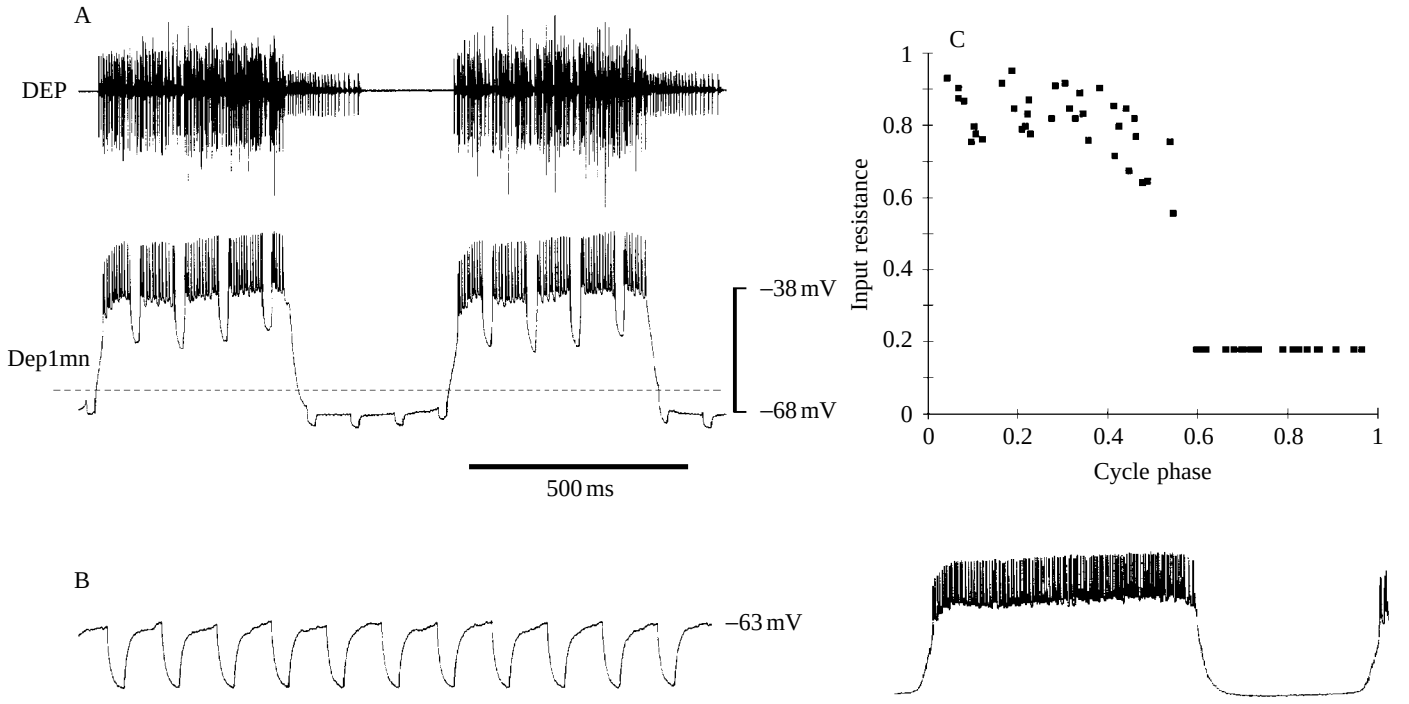

Fig. 7. Changes in motor neuron input resistance during the ventilatory cycle. The input resistance of this neuron was determined by the injection of −0.5 nA, 80 ms duration, hyperpolarizing current pulses during forward ventilation (A) and also during a pause in ventilation (B). The input resistance during ventilation was determined as a fraction of the 'rest' value during the pause and plotted against the time of application of the test pulse in the ventilatory cycle (C). The input resistance is lowest during the period when the motor neuron receives an inhibitory input, but is also lower than the reference ('rest') level during the depolarizing phase of the membrane potential oscillation. One cycle of the motor neuron burst is shown below the graph for reference. DEP, extracellularly recorded ventilatory motor pattern.

during forward ventilation, and the amplitude of these pulses was measured over 10 cycles of the ventilatory motor pattern (Fig. 7A). The input resistance was also determined during a pause in ventilation at a membrane potential of −63 mV, and this value was used as a reference in order to calculate the input resistance as a fraction of this 'rest' level during the pause (Fig. 7B). The relative value of input resistance was then plotted with respect to the phase of the ventilatory cycle when the test pulse was applied (Fig. 7C). During the hyperpolarized phase of the motor neuron's oscillation, the input resistance is reduced to approximately 20 % of the 'rest' value, in accord with the previous finding that the neuron receives a strong inhibitory synaptic input during this portion of the cycle (Simmers and Bush, 1983*b*). During the plateau phase, the input resistance is also lower than the rest value and averages approximately 85 % of the rest value. This observation is consistent with a conductance increase due to the inward current associated with a plateau potential underlying the motor neuron burst, although a portion of this conductance change may be due to the steady-state activation of other conductances at this relatively depolarized membrane potential. A similar result would also be obtained if there was a tonic chemically mediated excitatory input to the neuron when the CPG was active.

Modulation of plateau properties in ventilatory motor neurons

Plateau potentials have not been observed previously in any of the ventilatory motor neurons during pauses in ventilation (Simmers and Bush, 1983*b*; DiCaprio and Fourtner, 1984), and this finding has been confirmed in the present study. When the motor pattern stops spontaneously, plateau potentials cannot be triggered by depolarizing current pulses of up to 10 nA amplitude and 200 ms duration, even if these pulses are applied shortly after the cessation of the ventilatory rhythm. Longer-duration current pulses (300–1000 ms) could elicit sustained firing (Fig. 8), but the motor neuron returned immediately to its resting membrane potential when the current was turned off. Within the limits imposed by bridge imbalance, the depolarization caused by current injected appeared to be linearly related to the magnitude of the injected current. During ventilatory pauses, the current/firing relationship of the motor neurons was essentially linear (Simmers and Bush, 1983*b*). For example, the current/firing relationship for the Dep1 motor neuron shown in Fig. 8 was linear (r=0.99) with a slope of 25 spikes s^{-1} nA^{-1} for injected current of 1–10 nA. Plateau potentials also could not be elicited during pauses in ventilation if a motor neuron was hyperpolarized with various levels of intracellular current (1–5 nA) for 1–3 s before a depolarizing pulse was applied. Expression of plateau potentials in ventilatory motor neurons thus appears to be controlled by the ventilatory CPG, or by descending inputs to the CPG, as they can only be produced during the generation of the ventilatory motor pattern. This result was obtained for both forward and reversal motor neurons.

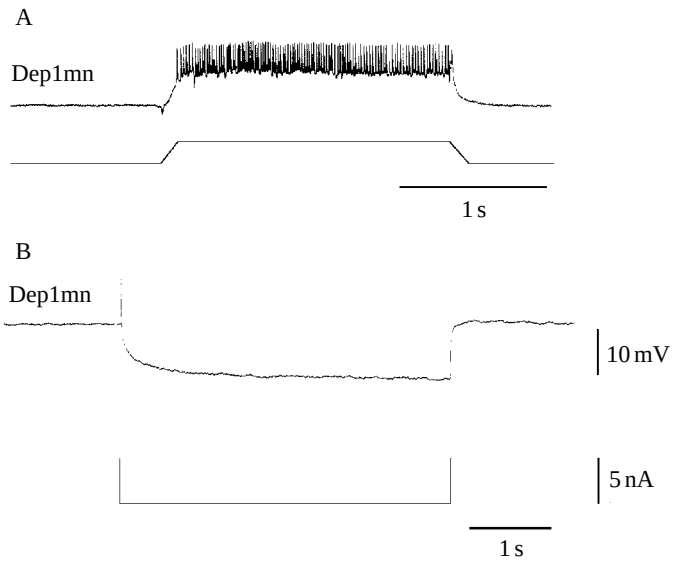

Fig. 8. Plateau potentials cannot be triggered in motor neurons when the ventilatory central pattern generator is not active. (A) Depolarizing current steps (lower trace) (1.5 s duration) of 1–10 nA were injected into a depressor group 1 motor neuron (Dep1mn, upper trace) (the same neuron as shown in Fig. 9) during a pause in the ventilatory rhythm. The current/firing relationship was linear over this current range (r=0.99) and the slope of the regression line fitted to the data was 25 spikes s^{-1} nA^{-1}. (B) During a complete pause in ventilation, long-duration hyperpolarizing current steps (lower trace) of 3–6 nA did not produce a sag voltage or any post-inhibitory rebound in this depressor motor neuron or other ventilatory motor neurons.

Possible presence of an I_h current in ventilatory motor neurons

Many motor neurons in rhythmic systems contain a hyperpolarization-activated depolarizing current, or I_h. While there is as yet no direct evidence from voltage-clamp experiments for this current in crustacean ventilatory motor neurons, their membrane potential oscillation during normal ventilation while plateau potentials are suppressed with applied hyperpolarizing current suggests that an I_h current may be present in these motor neurons.

Fig. 9A is an intracellular recording from a depressor Dep1 group motor neuron before and during the injection of a constant −1.1 nA current into the cell. At this level of injected current, the plateau potential is suppressed for the next three cycles of the motor pattern, but the amplitude of the subthreshold depolarizing oscillation progressively increases in amplitude. During the next (fourth) cycle, the oscillation amplitude has increased sufficiently to elicit two action potentials in the neuron, and an attenuated plateau is produced in the subsequent cycle. The motor neuron continues to burst, but only in alternating cycles of the motor pattern, and this behavior persisted for the remaining time that the neuron was held hyperpolarized. This behavior satisfies the graded burst-rate test for plateau potentials (Russell and Hartline, 1982), in that plateau-generating follower neurons would be expected to skip one or more

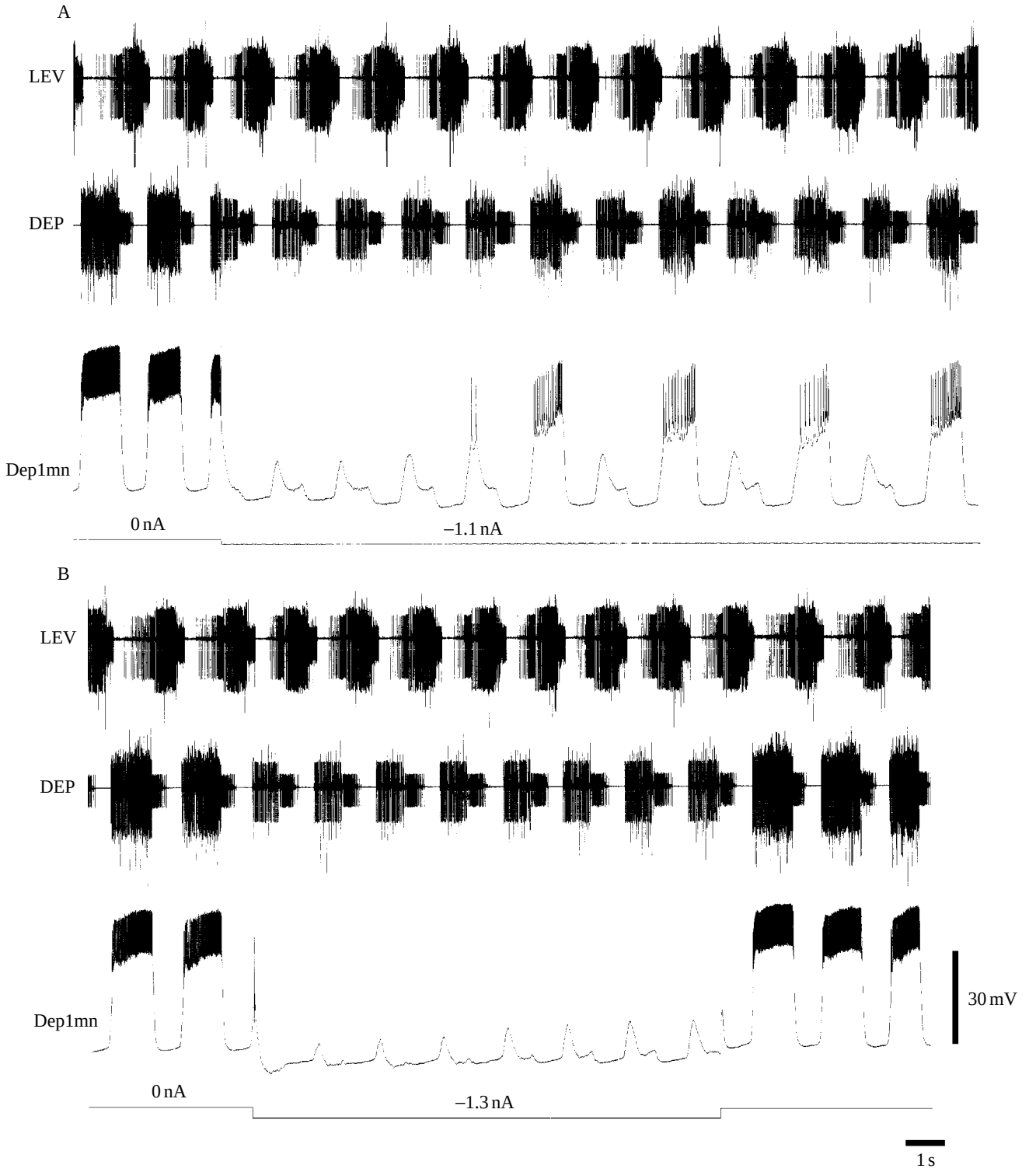

Fig. 9. The change in subthreshold membrane potential oscillation in ventilatory motor neurons during rhythmic central pattern generator (CPG) activity suggests the existence of a hyperpolarization-activated depolarizing current (an I_h current) in these neurons. (A) An intracellular recording from a depressor group 1 motor neuron (Dep1mn) during the injection of a −1.1 nA hyperpolarizing current (bottom trace). The plateau potential underlying the motor neuron burst is initially suppressed, but the depolarizing phase of the remaining subthreshold oscillation gradually increases in amplitude until the neuron generates a burst of action potentials in alternate cycles of the motor pattern. (B) A slightly larger-amplitude hyperpolarizing current (−1.3 nA) completely suppresses the plateau potential in the motor neuron. At this level of current, the neuron does not resume bursting and the gradual increase in the depolarizing phase of the membrane potential oscillation continues for the duration of the current step. The hyperpolarizing phase of the oscillation is due to a cyclic inhibitory input from the ventilatory CPG. DEP, extracellularly recorded ventilatory pattern from the depressor motor neuron; LEV, extracellularly recorded ventilatory pattern from the levator motor neuron.

bursts during injection of a hyperpolarizing current that did not completely suppress the plateaus.

If the hyperpolarizing current is increased to a level that completely inhibits the plateau potentials (Fig. 9B), the depolarizing phase of the subthreshold oscillation again increases in amplitude for each successive cycle after the onset of the current step. These observations are consistent with the presence of an I_h current in ventilatory motor neurons, as the maintained hyperpolarization in concert with the cyclic inhibition would result in the increased activation of such a current. Note also that the upward concave trajectory of the membrane potential after the peak of the depolarization is suggestive of a sag voltage resulting from activation of I_h at this membrane potential. Both a sag voltage during a step hyperpolarization and post-inhibitory rebound depolarization after the step would indicate a functional I_h current in the motor neuron. However, when the ventilatory rhythm is stopped, there is little or no rebound depolarization evident after the injection of a hyperpolarizing current step, and no sag voltage is observed in the membrane potential during the period of hyperpolarization (Fig. 8B). Therefore, the expression of this putative I_h current, like the inward current underlying the plateau potential, appears to be modulated by the ventilatory CPG.

Discussion

The formation of motor neuron bursts in the motor neurons in the crab ventilatory system had previously been attributed to the presence of a cyclic inhibitory input from the ventilatory CPG (Simmers and Bush, 1983*b*; DiCaprio and Fourtner, 1984). The data presented in the present study indicate that plateau properties are a major feature of these motor bursts. All of the ventilatory motor neurons exhibit large-amplitude (15–30 mV) oscillations in membrane potential. When a ventilatory motor neuron is held hyperpolarized with intracellular current injection, the large-amplitude oscillations in membrane potential are suppressed, and only a comparatively low-amplitude (5–8 mV) oscillation is observed in phase with the motor pattern (Fig. 1). This indicates the presence of an all-or-none threshold-dependent event (plateau) in the motor neurons. If a brief depolarizing current pulse is injected into a motor neuron while it is being held hyperpolarized, a large-amplitude all-or-none depolarizing plateau potential is elicited along with a burst of action potentials. This burst continues until it is terminated by the inhibitory input from the ventilatory CPG (Fig. 1B). Plateau potentials could be suppressed by maintained hyperpolarizing current injection in all of the forward motor neurons that were examined (Fig. 2)

Plateau potentials should be able to be terminated by the injection of hyperpolarizing current. In this system, hyperpolarizing current pulses could not terminate normal motor bursts during the expression of the ventilatory motor pattern (Fig. 3). Pulses applied during the middle of a motor neuron burst reset the motor pattern, but the motor neuron returned to the depolarized plateau level of membrane potential upon termination of the pulse. The retriggering of the plateau potential may indicate the presence of sufficient tonic depolarization to elicit a plateau potential after the hyperpolarizing pulse or, in view of the possible presence of an I_h current, post-inhibitory rebound could serve to trigger the plateau. When a hyperpolarizing pulse was injected close to the end of the normal burst period, the duration of the burst was decreased. However, in the light of the resetting of the ventilatory rhythm, the termination of the burst cannot be unambiguously attributed to the injected current because an inhibitory input from the CPG could also be present. In contrast, when plateau potentials were suppressed by the injection of constant hyperpolarizing current, plateaus triggered by a depolarizing pulse could be terminated prematurely by the injection of a hyperpolarizing pulse (Fig. 4). Identical results were obtained for all of the forward ventilatory neurons that were examined.

Plateau potentials in reversal motor neurons

The ventilatory CPG can produce two distinct motor patterns corresponding to forward and reverse ventilation, in order to reverse the flow of water through the branchial chamber. Correlated with this switch in motor pattern are changes in the amplitude and phasing of some ventilatory CPG interneurons (DiCaprio, 1989), frequency-modulating interneurons (DiCaprio and Fourtner, 1988) and the depolarization of the reversal switch interneuron (DiCaprio, 1990). In addition, the motor neurons that innervate the Lev2 and Dep2 muscle groups do not fire during reverse ventilation, but are replaced by a set of ‘reversal’ motor neurons that innervate these muscle groups (Young, 1975; Simmers and Bush, 1983*a*; DiCaprio and Fourtner, 1984). The membrane potential of reversal motor neurons oscillates in phase with the forward ventilatory motor pattern, but the membrane potential is always subthreshold for the production of action potentials.

If a brief depolarizing current pulse is injected into a reversal motor neuron during forward ventilation, a plateau potential is triggered in the neuron that is then terminated by the inhibitory input from the CPG (Fig. 5). These triggered plateaus may also be prematurely terminated by the injection of a hyperpolarizing current pulse before the onset of the CPG-derived inhibitory input (Fig. 6). Plateau potentials can therefore be triggered in reversal motor neurons during forward ventilation and, given the similarity between the triggered burst and the bursts during reverse ventilation (Fig. 5B), it is likely that plateau potentials are normally produced in these neurons during reverse ventilation. Owing to the random and transient nature of episodes of reverse ventilation, it was not possible to determine whether plateaus in reversal motor neurons could be suppressed by hyperpolarizing current during reverse ventilation, or whether plateau potentials could be triggered in forward motor neurons that are normally silent during reverse ventilation.

Motor neuron input resistance during the ventilatory cycle

The generation of a plateau potential by a motor neuron should be accompanied by an increase in the membrane conductance during the plateau due to the activation of the inward current that underlies the plateau. This change in

conductance was assessed by measuring the change in input resistance of ventilatory motor neurons during the ventilatory cycle relative to the input resistance measured during pauses in ventilation. Although it cannot be assumed that the motor neurons are not influenced by tonic synaptic input during pauses in ventilation, their membrane potentials are relatively hyperpolarized (−60 to −70 mV) during ventilatory pauses and they are not tonically active. The input resistance of ventilatory motor neurons decreases markedly during the hyperpolarized phase of their oscillation, which has been shown to be due to a strong inhibitory synaptic input. During the plateau phase of their oscillation, the input resistance is also reduced compared with the resistance measured during a pause (Fig. 7). This finding is consistent with the activation of a regenerative inward current underlying the plateau, but it is not necessarily conclusive evidence for such a current. The same result would be obtained if the motor neurons were receiving a tonic excitatory input along with the cyclic inhibition in order to form the motor neurons bursts, although this scheme would not lead to suppression of the depolarizing oscillation during maintained hyperpolarization, as has been observed in these experiments. In addition, some of the observed conductance decrease is probably due to the conductance changes associated with the generation of action potentials or any additional net steady-state conductance increase at the depolarized membrane potential level obtained during the motor burst. It was not possible to determine accurately the input resistance during a pause in ventilation when the neuron was held depolarized to the plateau level owing to the nonlinearity of the electrodes and the resultant bridge imbalance at different levels of sustained current injection.

Absence of plateau properties in ventilatory neurons during pauses in ventilation

It was not possible to trigger plateau potentials in ventilatory motor neurons when the ventilatory CPG was not producing a motor pattern. Injection of depolarizing current pulses of up to 10 nA in amplitude during a pause did not trigger plateau potentials in any of the motor neurons tested. Altering the resting membrane potential of these neurons with depolarizing or hyperpolarizing current before the injection of a depolarizing current pulse also did not enable the triggering of a plateau potential. Depolarizing current pulses injected immediately (500–800 ms) after the ventilatory rhythm had stopped were also ineffective in triggering a plateau. Injection of longer-duration current steps resulted in tonic firing of a motor neuron, with a linear relationship between current amplitude and action potential frequency. The change in membrane potential also appeared to vary linearly with (low-amplitude, 1–3 nA) injected current, although this observation is not certain given the potential for bridge imbalance during the current injection and the high probability of bridge imbalance at high current levels due to nonlinearities in the microelectrode.

The expression of plateau properties would therefore appear to be dependent upon influences from the ventilatory CPG or possibly from descending inputs to the motor neurons that also activate the CPG. The modulation of plateau properties has been observed in several other systems where various neuromodulators or neurotransmitters can induce or otherwise modulate the expression of plateau properties. For example, plateau properties in pyloric neurons of the stomatogastric system can be induced by activation of a modulatory interneuron (Dickinson and Nagy, 1983), serotonin (5-HT) can induce plateau properties in a gastric motor neuron (Kiehn and Harris-Warrick, 1992) and octopamine can induce plateau bursts in locust flight and respiratory neurons (Ramirez and Pearson, 1991). In vertebrate systems, plateau properties can be induced in cat motor neurons by the noradrenergic precursor L-DOPA (Conway *et al.* 1988) and the serotonin precursor 5-HTP (Hounsgaard *et al.* 1988) and by 5-HT in turtle spinal motor neurons (Hounsgaard and Kiehn, 1989). The agent or mechanism responsible for the induction of plateau properties in the crab ventilatory system is as yet unknown.

Is an I_h current present in ventilatory motor neurons?

The presence of a hyperpolarization-activated inward current, or I_h, is often associated with neurons that produce plateau potentials (Arbas and Calabrese, 1987; Hounsgaard and Kiehn, 1989), and the post-inhibitory rebound depolarization produced by I_h activation may serve to trigger a plateau potential. Ventilatory motor neurons appear to possess an I_h current because, when a motor neuron is held hyperpolarized at a level that prevents the generation of plateau potentials, a transient depolarizing potential is observed immediately following the period when the neuron is (further) inhibited by the CPG. This depolarizing potential increases in amplitude during successive cycles of the motor pattern, as would be expected if this behavior were due to a hyperpolarization-activated inward current. The membrane potential of the neuron also follows an upward concave (sag-like) trajectory after the depolarization, which is also to be expected from the activation of an I_h current. Like the inward current responsible for the plateau potential, however, this current only appears to be active while the motor pattern is being expressed because, during pauses in ventilation, a sag voltage was not observed during the injection of a hyperpolarizing current and no rebound depolarization was seen when the pulse was terminated (Fig. 8B). Simmers and Bush (1993*b*) observed a slight rebound depolarization in ventilatory motor neurons during pauses in ventilation after the termination of an injected hyperpolarizing current in addition to a sag voltage during the current injection. However, their records indicate that there was a significant amount of tonic activity in several ventilatory motor neurons and also possibly some weak cyclic activity in the motor output (Simmers and Bush, 1983*b*; their Fig. 5). The amplitude of the rebound response that they observed was of the order of 2–3 mV, which is less that the depolarization observed after the release from inhibition observed here (Fig. 9A; approximately 10 mV) when the ventilatory motor pattern was being fully expressed. The results of Simmers and Bush (1983*b*) therefore support the

hypothesis that the ventilatory motor neurons possess an I_h current, but that this current is only fully functional when the ventilatory CPG is active.

Plateau potentials and the ventilatory motor pattern

The ventilatory motor neurons thus satisfy several of the criteria that have been proposed for the demonstration of plateau potentials by Russell and Hartline (1982); namely, the trigger, termination, threshold, all-or-none, regenerative, trajectory acceleration, graded burst-rate and the critical hyperpolarization tests. In addition, measurement of the input resistance of these motor neurons during the ventilatory cycle indicates a decrease in input resistance (conductance increase) during the plateau, which is consistent with the presence of a voltage-dependent inward current during the plateau. The timing of motor neuron bursts in the crab ventilatory system would therefore be determined by the cyclic synaptic inhibition from the ventilatory CPG, as has been proposed previously (Simmers and Bush, 1983*b*), which, in conjunction with a rebound depolarization due to a hyperpolarization-activated inward current, results in the initiation of a plateau potential and a burst of action potentials. The presence of a plateau potential removes the need for the CPG to supply an excitatory drive to the motor neurons for the duration of the burst, although the specific structure of individual motor neuron bursts may be determined by the kinetics of the plateau current or, additionally, by other inputs from the CPG.

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