Driver potentials and the organization of rhythmic bursting in crustacean ganglia

Jack A. Benson and Ian M. Cooke

The organization into bursts of action potential firing is a characteristic of the neurons of many groups of animals. In the crustacean cardiac and stomatogastric ganglia, burst generation and perhaps also endogenous rhythmicity result from the production of slow, Ca^{2+}-dependent, non-propagated depolarizations known as driver or plateau potentials. At least some neuromodulatory inputs to these ganglia have their effects by acting on these potentials, and driver potential formation appears to be an essential aspect of non-impulse-mediated synaptic co-ordination in these ganglia.

In the Crustacea and some other arthropods such as Stomatopoda and *Limulus*, the co-ordinated and periodic muscular contractions of the heart and foregut are brought about by bursts of impulses from neurons located in small, semi-autonomous ganglia. For example, the cardiac ganglion of lobsters and crabs consists of nine neurons[1], which in isolation produce the rhythmic, co-ordinated impulse bursts controlling the frequency and amplitude of the heartbeat. The more complex contractions of the foregut are controlled by the stomatogastric ganglion which contains some 30 identified neurons[2] of which 14 comprise the pyloric sub-network to be discussed here. The output from the motor neurons of both the cardiac and the stomatogastric ganglia is made up of bursts of action potentials which recur at regular intervals[3] and which display relatively fixed phase relations among the different motor neurons[1,2]. In this review, we will examine the mechanism by which action potentials are organized into bursts, how this mechanism contributes to rhythmicity and to interneuronal co-ordination among these neurons, together with some of the ways in which neurohormones and input from CNS neurons might sustain or modulate their activity.

Driver potentials

The electrical activity recorded in the soma of a crustacean cardiac or pyloric neuron, whether a pacemaker or a motor neuron, takes the form of a square-shaped depolarization, often called a 'plateau potential'[3]. This potential is surmounted by attenuated action potentials as well as electrical and chemical synaptic potentials from other neurons within the ganglion (Figs 1 and 2A).

Working on the cardiac ganglion of the crab *Portunus sanguinolentus*, Tazaki and Cooke made the following observation[4–6]. When tetrodotoxin (TTX) is applied to the cardiac ganglion, the action potentials are suppressed, but it remains possible to evoke, with a small injected current pulse, a slow, regenerative depolarization (Figs 1, 3 and 5). This was called a 'driver potential' because it is the driving force underlying burst formation, and in the absence of TTX it constitutes the characteristic square plateau potential seen in recordings from the soma. TTX-resistant oscillations have now been observed in the pyloric neurons of the stomatogastric ganglion in special circumstances which we shall discuss below (Refs 7 and 8; Nagy, F. and Benson, J. A. unpublished observations). These oscillations appear to be rhythmically generated driver potentials, and it has been possible to evoke driver potentials with current pulses (Fig. 2E and F).

In both the cardiac and stomatogastric neurons, the driver potentials are regenerative responses. Providing that the appropriate ion channels are capable of activation, driver potentials appear to be elicited in response to any depolarizing influence regardless of its source (a 'pacemaker potential', a chemical synaptic potential, an antidromically propagating action potential, or the spread of depolarization from the driver potential in another neuron of the ganglion by electrotonic synapses).

Ionic mechanism

What is the ionic basis of the driver potential? The first clues to the nature of the currents underlying the cardiac and pyloric neuron driver potentials came from channel block and ion substitution experiments[6,10]. In the lobster cardiac ganglion, some individual motor

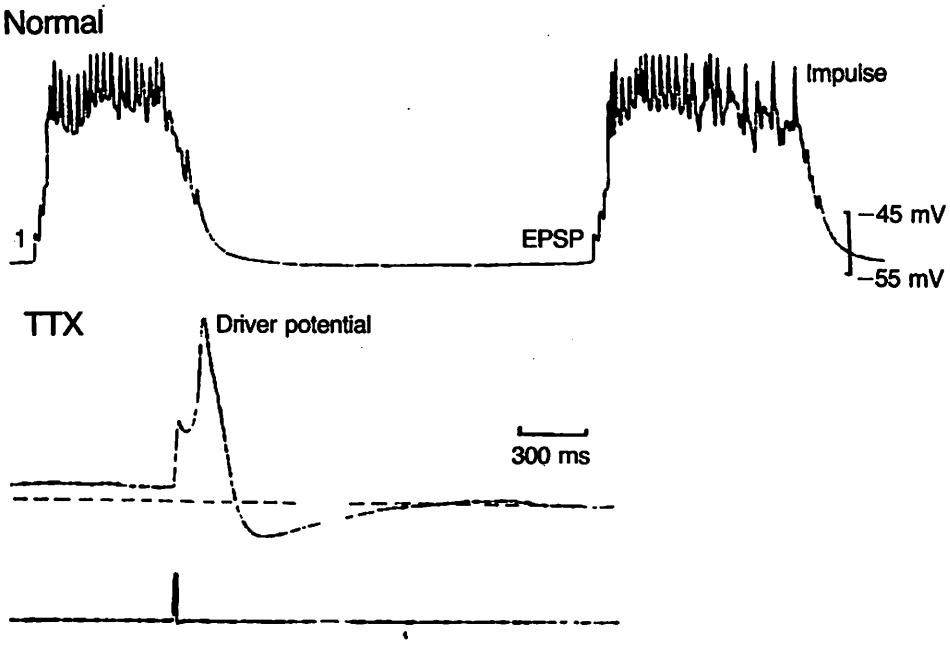

Fig. 1. *Spontaneous activity and an evoked driver potential in a lobster cardiac ganglion motor neuron – intracellular recording from Cell 1 of* Homarus amerianus *(not ligatured, see diagram in Fig. 5). Rhythmic bursts of activity, as seen in the upper trace, continue for 10 h or more after isolation of the ganglion. Excitatory postsynaptic potentials (EPSPs) produced by action potentials of posterior small neurons begin each burst; they lead to a driver potential on which are superimposed the electrotonically decremented action potentials generated at the axonal trigger zone (see Figs 5 and 6), as well as continuing EPSPs; the latter sustain the amplitude and duration of the underlying driver potential. Perfusion with tetrodotoxin (TTX) (3×10^{-7} M) leaves the ganglion quiescent, and then a brief depolarizing current pulse evokes a driver potential (see Figs 3 and 5 for other examples).* (Adapted from Ref. 13.)

neurons can be electrically isolated from the other neurons in the ganglion by placing ligatures around the ganglionic trunks. This has made possible voltage clamping of the soma and proximal axon to measure the membrane currents directly[11]. The depolarizing current contributing to the driver potential in both the cardiac and the pyloric systems is carried by Ca^{2+} ions[6,10,11]. The amplitude of the driver potential is dependent on extracellular Ca^{2+} concentration and the inward current is blocked by Mn^{2+}, Cd^{2+} and Co^{2+} ions which block Ca^{2+} channels (Fig. 3). The voltage-clamp measurements of this Ca^{2+} current show that it is much slower both in terms of onset and relaxation with depolarization than the analogous current, I_{Ca}, of mollusc neurons (Fig. 4). The inward current shows a partial inactivation with sustained depolarization occurring with a time course of tens of milliseconds[11]. Since this time course is independent of the clamp potential, a calcium-mediated inactivation process may be involved. K^+ channels are thought to contribute to the active repolarization which terminates the driver potential[11]. Extra- or intra-cellular tetraethylammonium (TEA), a K^+-channel blocker, prolongs driver potentials (Fig. 4)[6]. The voltage clamp reveals three different K^+ currents in the cardiac neurons[11], all of which may contribute to repolarization. These currents are analogous to the fast transient (I_A), delayed (I_K) and calcium-activated (I_c) K^+ currents of molluscan neurons and are probably responsible for the biphasic after-hyperpolarization observed in the cardiac neurons (Fig. 3)[5]. The relative contributions of Ca^{2+}-current inactivation and K^+-current activation to the driver potential termination are as yet unresolved.

A driver potential can thus be described in part as an endogenous, regenerative, Ca^{2+}-mediated depolarization of duration 200–600 ms and amplitude 20–40 mV, with some contribution by one or more K^+ currents to its repolarizing phase. While driver and plateau potentials share common activation properties, plateau potentials differ in having an indefinite duration.

Topographical localization

Another highly distinctive feature of the driver potential of lobster and crab cardiac ganglia[12,13] (and, we speculate, of the pyloric system as well) is that it is not a propagated potential. All the evidence is consistent with the hypothesis that the driver potential is generated in and confined to a region including a proximal segment of the axon (and, in lobster, the soma) which does not conduct action potentials. Tazaki and Cooke used fine threads to ligate the cardiac axons at various distances from their somata. By this means, they were able to show that action potentials arise at a 'trigger zone' up to several millimeters from the soma, and that driver potentials could be evoked in the somata thus separated physically from the distal axonal regions as well as from most synaptic input.

In Fig. 5 are shown the evoked, regenerative potentials recorded from the somata of the anterior cardiac

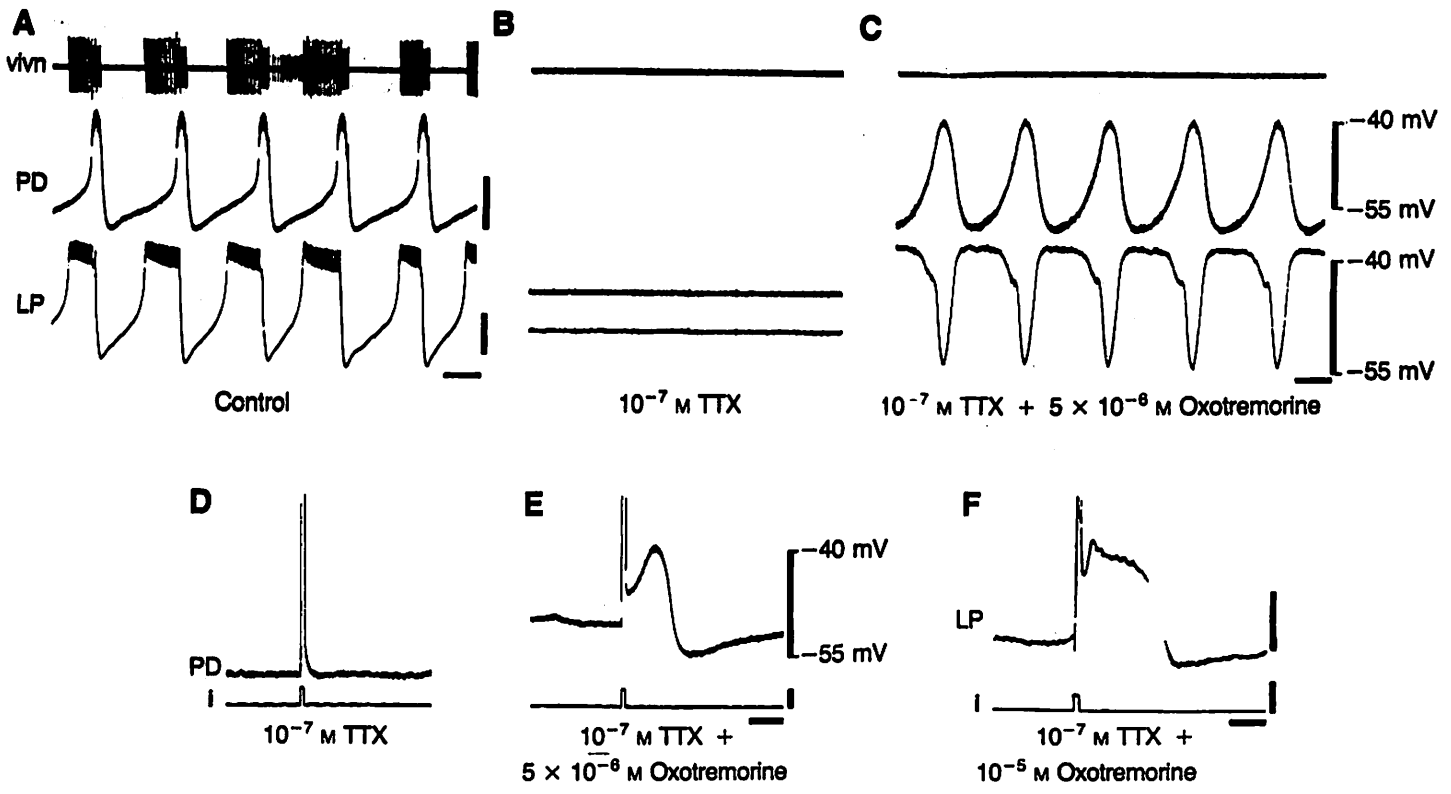

Fig. 2. *Restoration of rhythmical driver potential generation and plateau potential capability by oxotremorine in stomatogastric neurons of the cape lobster,* Jasus lalandii *– intracellular recordings from two motor neurons (pyloric dilator, PD, and lateral pyloric, LP), and an extracellular recording from the ventro-lateral ventricular nerve (vlvn) carrying their axons. All records were made in the presence of 2 × 10⁻⁶ M picrotoxin (an inhibitor of some synapses) to simplify analysis.* **(A)** *With input from CNS ganglia intact, plateau potentials in LP and PD recur rhythmically; they are surmounted by deflections representing the electrotonically decremented action potentials generated at a distance in their axons.* **(B)** *Addition of 10⁻⁷ M tetrodotoxin (TTX) silences the network. Both PD and LP have high resting potentials. TTX inhibition of CNS input leaves the PD neuron incapable of responding to a depolarizing current with a driver potential* **(D)**. **(C)** *After the addition of 5 × 10⁻⁶ M oxotremorine, rhythmic driver potentials appear spontaneously in the PD neuron, and, properly phased, in the LP neuron; no action potentials are present due to the presence of TTX.* **(E)** *After the addition of oxotremorine (in TTX), the PD neuron is capable of responding to a brief current pulse with a driver potential (compare with* **D**). **(F)** *The LP neuron responds to a brief depolarizing pulse with a typically longer driver potential after the addition of oxotremorine.* (Adapted from Ref. 9.)

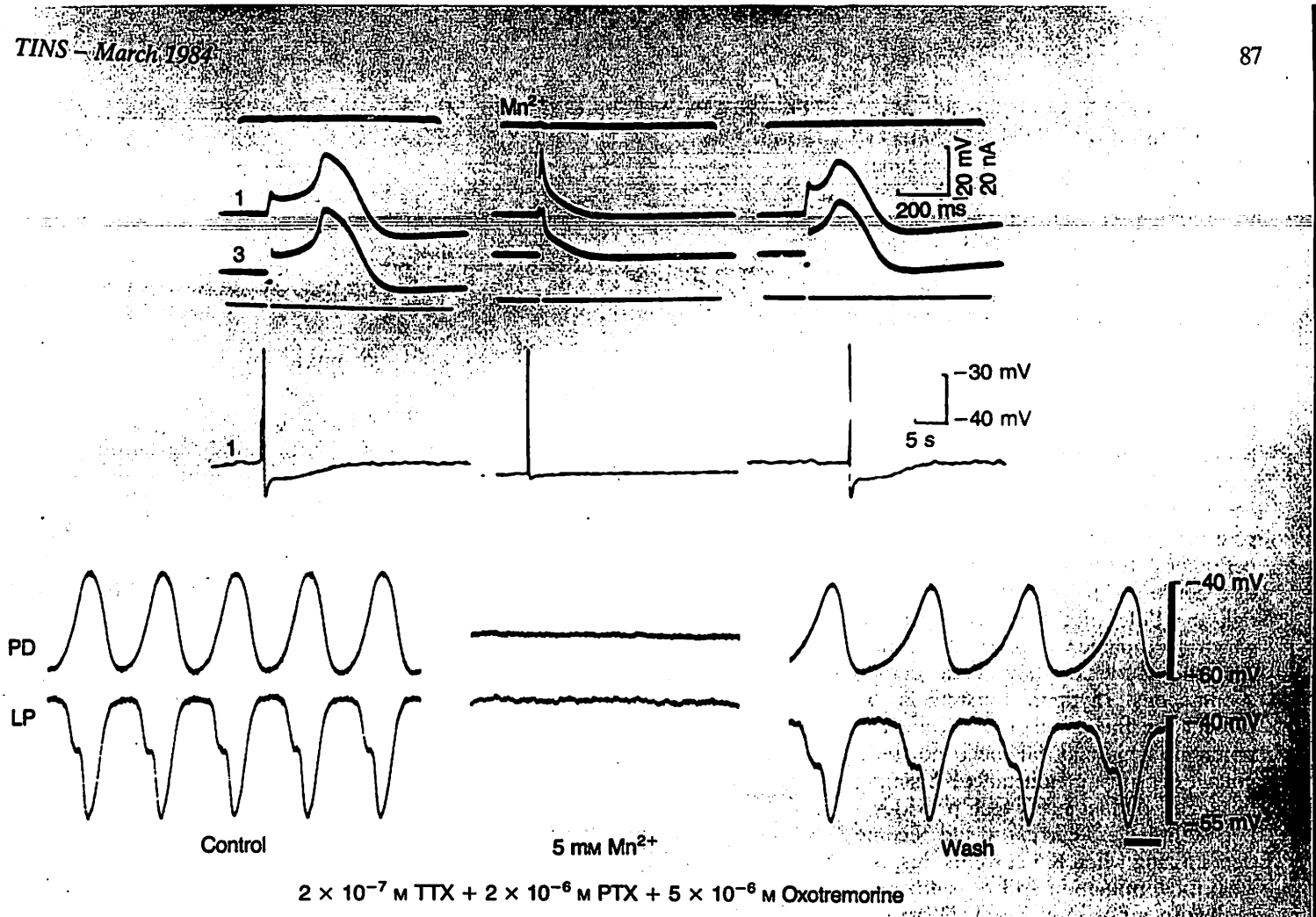

Fig. 3. *Inhibition of driver potentials by Mn^{2+}.* **(Top)** *Intracellular recording from two crab (*Portunus sanguinolentus*) cardiac ganglion motor neurons showing electrotonically co-ordinated driver potential responses to a brief depolarizing current injected through the electrode in Cell 3, before (left) and after (right) recovery from the introduction of 4 mM Mn^{2+}; in the presence of Mn^{2+} (middle), regenerative responses are blocked (note the larger current amplitude). Traces below are pen-writer records of the same responses of Cell 1 at a slow time base to show the biphasic hyperpolarizing after-potentials which follow driver potentials.* (From Ref. 6.) **(Below)** *Simultaneous intracellular recording from identified motor neurons of the pyloric network of the cape lobster (*Jasus lalandii*) stomatogastric ganglion showing spontaneous rhythmic driver potential generation by the pyloric dilator (PD) neuron (upper beam) and by the lateral pyloric (LP) neuron in the presence of 2×10^{-7} M tetrodotoxin (TTX), 2×10^{-6} M picrotoxin (PTX), and 5×10^{-6} M oxotremorine. Addition of 5 mM Mn^{2+} reversibly inhibits all phasic activity of the network (middle). The PD neuron shows a normal resting potential, the LP neuron shows a depolarized potential corresponding to the plateau level. (See also Fig. 2 and Refs 7 and 8.)* (Adapted from Ref. 9.)

neurons of the lobster (in both cases, the identified Cell 1) on which ligatures have been placed 1.6 mm and 200 μm from the soma. Photomicrographs of the same cells injected with Lucifer Yellow are also shown in Fig. 5. When the ligature was made more distant, the evoked potential consisted of a driver potential surmounted by a train of action potentials representing the burst of axonal action potentials. A ligature placed between the axonal 'trigger zone' and the proximal axon and somal region sustaining the driver potential allowed a driver potential to be evoked in isolation from any form of intercellular interaction.

Burst formation

Fig. 6 is a schematic representation of the functional regions of a cardiac motor neuron. We think it is likely that the pyloric neurons are similarly organized, but there is less evidence for this. The topographical relationship of the soma/proximal axon that generates the driver potential to the narrower distal axon that generates the action potential provides an appropriate arrangement for the driver potential to serve as a source of depolarizing current (a 'driving force') for the production of a burst of action potentials which then propagate to the muscles to produce a discrete contraction. After a driver potential has been evoked, a depolarizing current spreads away electrotonically from the cell body along the proximal axon to the neighboring more distal axonal region (the trigger zone) which is provided with the voltage-sensitive Na^+ channels necessary for action potential production. It is worthwhile noting that these bursts themselves have an internal temporal structure which arises from the interaction of the driver potential and the incoming synaptic activity from pacemakers and other bursting motor neurons in the cardiac ganglion. During normal activity in TTX-free saline, incoming excitatory postsynaptic potentials (EPSPs) not only initiate driver potentials but also prolong them and augment their amplitude (Fig. 1).

Spontaneous rhythmicity

So far, we have discussed the central part played by driver potentials in burst formation and action potential patterning as if these functions were entirely separate from the rhythm-generating mechanism. Rhythmicity is an endogenous property of the cardiac and stomatogastric ganglia, and it appears to arise, at least in part, from the properties of the driver potential[4,14]. Driver potentials exhibit a long, relatively refractory period during which the activation threshold is elevated[5,13]. This property, in combination with an activation threshold, provides the possibility for endogenous rhythmicity in a neuron. Given any form of tonic depolarization (a depolarizing 'pacemaker potential', a tonic bombardment of synaptic input, a continuous exposure to a depolarizing

neurohormone, or even random, spontaneous firing of action potentials), depending on the intensity and constancy of this activity the neuron will generate a driver potential upon sufficient recovery from the previous driver potential, thus creating a burst of action potentials and an intervening pause, until initiation by the tonic depolarizing influence again overcomes refractoriness.

In the cardiac ganglion, the importance of driver potential characteristics in the control of spontaneous rhythmicity is demonstrated by the effects of brief stimuli which elicit or suppress the occurrence of a burst. They 'reset' the phase of bursting without changing its period or pattern[14]. Given the capability of a neuron to produce a driver potential, the difference between a neuron which is capable of burst generation only and one which shows endogenous, spontaneous, rhythmic bursting is the presence in the latter of a 'pacemaker' potential. This may but need not be a voltage-sensitive conductance. A simple 'leakage' conductance which provides a persistent inward current would become depolarizing when K^+ currents, responsible for termination and repolarization of the driver potential, decay. When this depolarization exceeds the threshold for the initiation of a driver potential, the next burst is produced. Among both the cardiac and the pyloric motor neurons, there are some neurons capable of endogenous rhythmicity and others which, although they produce driver potentials, appear to be without pacemaker characteristics[1–4]. In the normally functioning cardiac ganglion, action potentials occur first in the four posterior small neurons and produce depolarizing synaptic potentials in the five large motor neurons. These in turn, either directly or as a result of initiating an axonal action potential, initiate the large-cell driver potentials[4,10,12,13]. However, in the absence of synaptic input, conduction via electrotonic pathways of the small neuron driver potentials is adequate to initiate the driver potentials of the other neurons[5]. Thus, co-ordinated action potential bursts of the motor axons (and thereby an effective heartbeat) are assured by a redundancy of depolarizing influences, any of which suffice to initiate a driver potential. The stomatogastric ganglion is more complex, involving a system of co-ordination in which driver potential production appears to be essential for graded, chemically mediated control of phase relations among the various burst cycles (Refs 7–8, 15; Nagy, F. and Benson

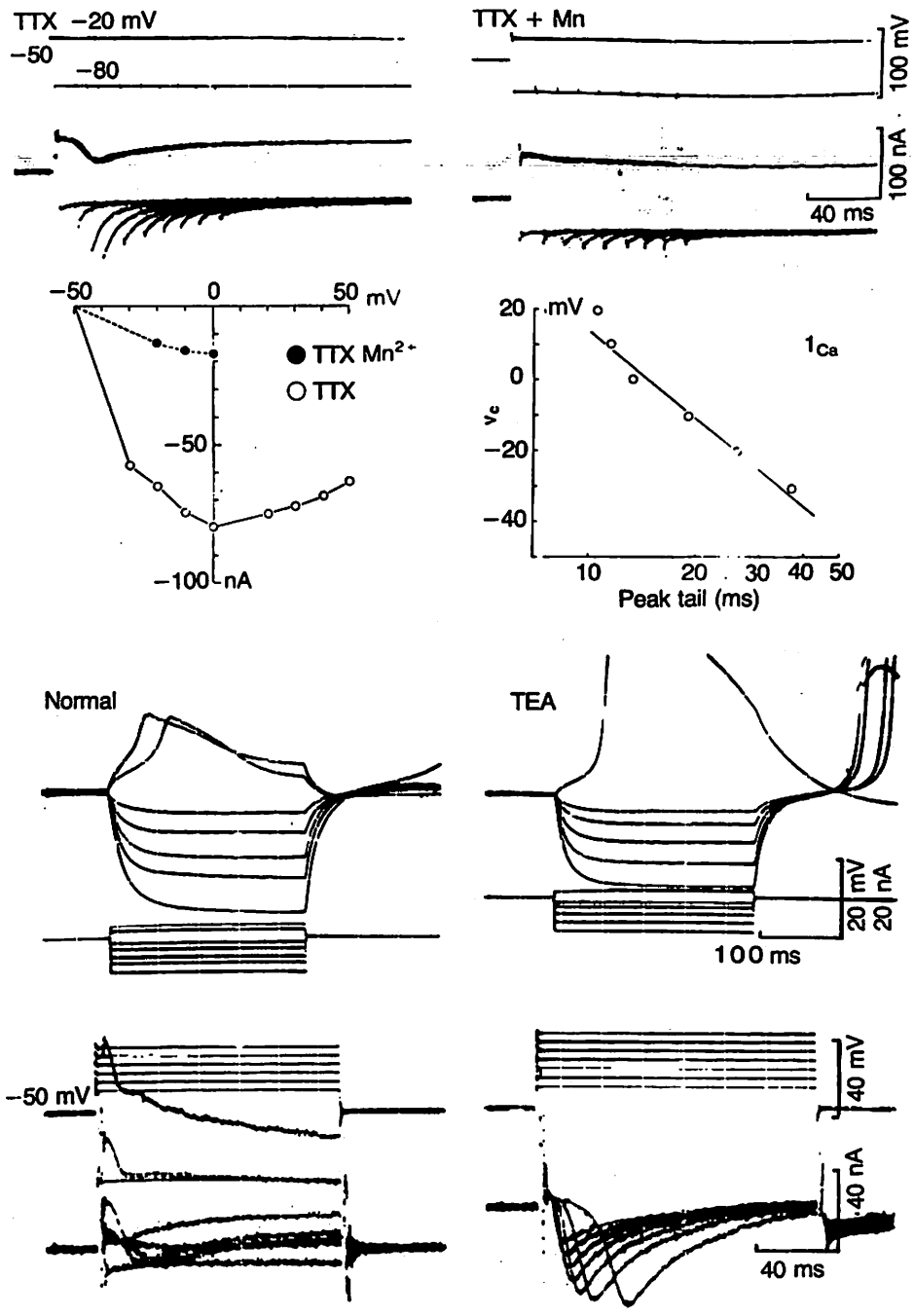

Fig. 4. *Voltage-clamp analysis of lobster cardiac ganglion motor neurons. A ligature isolates the soma and proximal axon of Cell 1 or 2. Separate current-passing and voltage-recording electrodes are inserted into the soma. In all oscilloscope frames, the upper beam records voltage and the lower beam records current; seven or more sweeps are superimposed in each frame. The upper frames shows inward 'tail currents' produced by stepping the membrane potential from the standard holding potential of −50 mV to −20 mV and then, at a different time for each sweep, to −80 mV (to eliminate competing outward currents). In one sweep, the depolarizing step is not given. The envelope formed by the downward peaks describes the magnitude and time course of inward conductance during depolarization held for about 100 ms. It is attributed to Ca^{2+} since it is recorded in the presence of 3×10^{-7} M tetrodotoxin (TTX) and is blocked by the addition of 45 mM Mn^{2+} to the saline (upper, right frame). Graphs show the amplitude of the peak tail currents observed following depolarizations to a range of values (−30 to +50 mV) and the time to the peak tail current versus depolarizing command. They show that a minimum of 10 ms is required for full activation of inward conductance. The frames immediately below show membrane potential responses to currents of a neuron ligatured at 200 μm (as in Fig. 5) in normal saline and after the addition of 50 mM tetraethylammonium (TEA) has blocked much of the outward current. In TEA, the membrane potential can become overshooting; it went off the oscilloscope face in this frame. Voltage-clamp records (lowest frames) show a prominent outward current component analogous to molluscan I_A and a later outward current (I_K); both are reduced by TEA (lower, right frame), revealing the slow inward current.* (Adapted from Ref. 11.)

Intercellular co-ordination

In both the cardiac and stomatogastric ganglia the participation of driver is important for interneuronal phase control and the overall co-ordination of the motor output from these ganglia.

interconnected by electrotonic pathways. Passive membrane properties and cell geometry result in the filtering in these pathways of rapid electrical responses (such as action potentials) and effective conduction of slow potential changes such as pacemaker and driver potentials[16]. In a TTX-treated crab ganglion, experimental initiation of a driver potential in any of the neurons leads, by electrotonic conduction of the depolarization, to driver potential responses in all other cells[5].

In stomatogastric ganglia, driver and plateau potentials may play a more complex and interesting role: not only are certain cells electrotonically coupled, but many form reciprocal, chemically-mediated, inhibitory synapses. These synapses have been shown not only to operate by well-known impulse-mediated mechanisms, but also in the range of values represented by pacemaker and plateau potentials to function tonically with graded release of transmitter under the control of membrane potential[7,8,15]. It is the effectiveness of plateau poten-

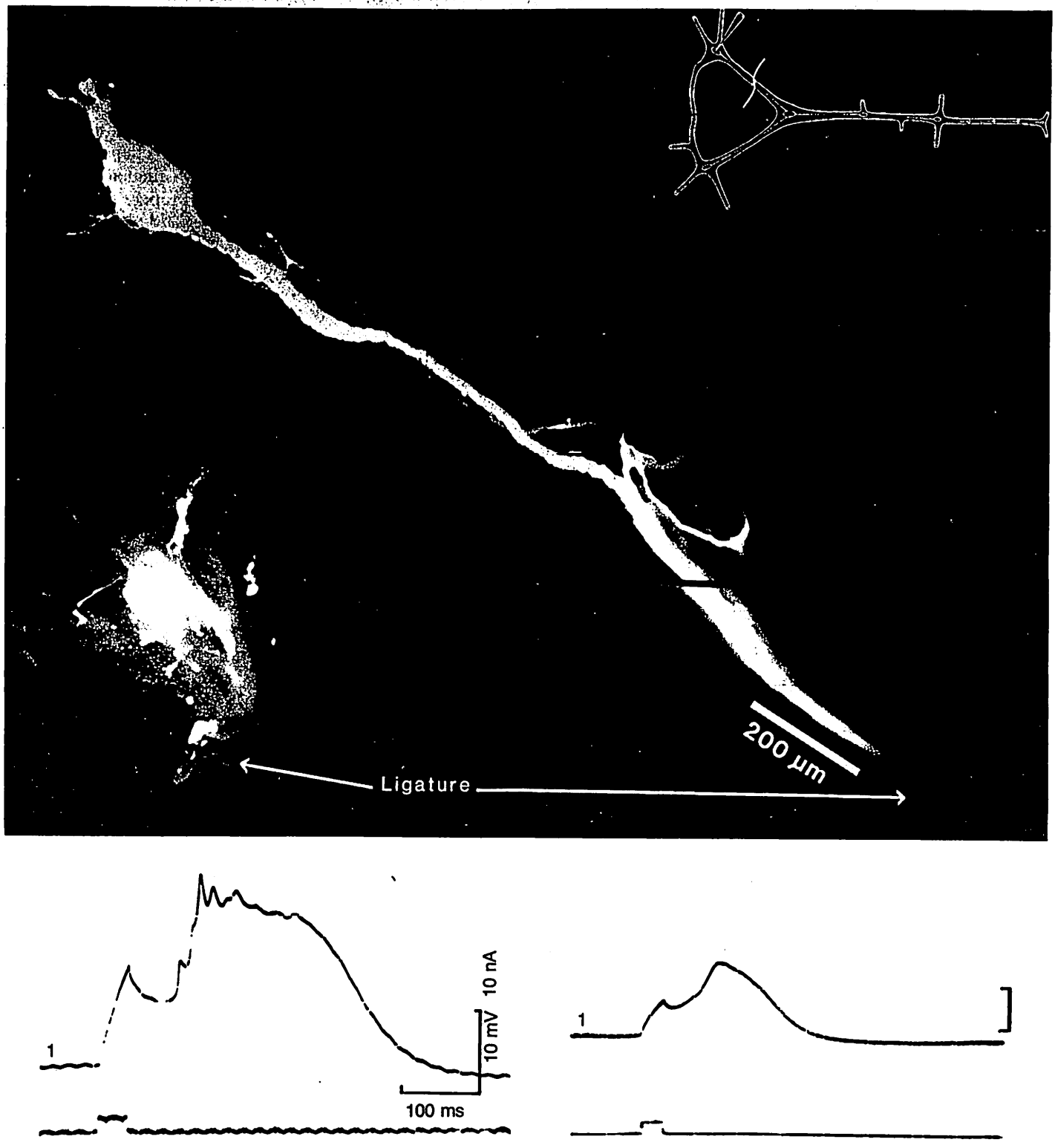

Fig. 5. *Topographic localization of driver potential and action potential initiation (trigger) zones in lobster cardiac ganglion motor neurons. An anterior neuron (Cell 1, see inset diagram) was ligatured at a distance of 1.6 mm from the soma (above) and, in another preparation, at 200 µm from the soma. Recordings from the somata of these same neurons (below) show a driver potential with superimposed action potentials (left), since the ligature is sufficiently distant to include the axon generating the action potential; these recordings also show a driver potential without inflections, in the closely ligatured cell, since the ligature has excluded the impulse-generating axon as well as collaterals on which synapses occur. After recording, the neurons were injected with the fluorescent dye Lucifer Yellow and*

tials in causing release of inhibitory transmitter that is apparently largely responsible for the appearance of properly phased, complete patterning of pacemaker and plateau potentials among the pyloric neurons in the presence of TTX (Figs 2 and 3)[7,8,15]. Clearly, non-impulse-mediated synaptic transmission is important in forming the complexly phased action potential burst patterns in this ganglion. In normal functioning, impulse-mediated synaptic transmission also participates.

Enhancement by neurohormones

Several putative crustacean neurohormones have been shown to modulate the activity of the cardiac and pyloric neurons. Two of these compounds have pronounced effects on driver potential production in the TTX-treated cardiac ganglion.

Proctolin, a pentapeptide which is released by neurohemal structures called pericardial organs, induces rhythmic driver potentials in the lobster cardiac ganglion by acting directly on the large motor neurons. Proctolin appears to reduce a resting conductance[17]. It remains to be seen whether it also acts on the driver potential currents directly.

Dopamine, also a neurohormone in Crustacea, increases the frequency and duration of bursts in the crab cardiac ganglion and increases the number of action potentials per burst[18]. By means of the ligature technique and selective application of dopamine to the neurons thus electrically isolated, it has been shown that dopamine selectively increases the amplitude and duration of driver potentials in the small pacemaker neurons[18].

CNS control over burst-forming capabilities

The cardiac ganglion of crabs and lobsters maintains its rhythmic activity for long periods when isolated from the CNS. In contrast, the lobster stomatogastric ganglion often falls silent when the stomatogastric nerve, which connects it to the oesophageal ganglion and the rest of the CNS, is severed. Many of the neurons cannot be induced to exhibit driver or plateau potentials by injection of current pulses in the non-cycling ganglion, but do so after stimulation of the CNS input nerve[3,7,8,19,20]. Thus, the ability of these neurons to generate burst-forming potentials is under CNS control.

The input nerve has been shown to include fibers of identified dopaminergic[21] and putatively cholinergic neurons[22,23] located more centrally. The effects of input nerve stimulation are imitated by application of acetylcholine or of muscarinic receptor agonists (for example oxotremorine, see Fig. 2) or of dopamine[7,8]. Further, in a TTX-treated, quiescent ganglion, application of these agents leads to the appearance of rhythmic driver potentials in certain neurons, and of slow potentials of roughly the same form and in the same phase relations as those normally underlying the bursting behavior in the other pyloric neurons. Non-impulse-mediated synaptic transmission, discussed earlier, is undoubtedly involved. With both oxotremorine and dopamine, elicitation of a driver potential in response to a brief depolarizing pulse has been observed in the presence of TTX[8,9]. The appearance of driver potential responses precedes the development of spontaneous cycling following the introduction of oxotremorine (Fig. 2).

For stomatogastric neurons, the application of voltage-clamping techniques is impractical. Available observations are consistent with postulating the Ca^{2+} dependence of driver and plateau potentials. Uncertainties of interpretation arise because the same conditions necessary to demonstrate Ca^{2+} dependence also block chemically mediated synaptic transmission. In preparations in which initiation of driver and plateau potentials is sustained by dopamine or oxotremorine, activity ceases in saline having low Ca^{2+} and high Mg^{2+}, or containing the Ca^{2+}-channel antagonists Mn^{2+} or Cd^{2+}. Thus, it seems likely that the driver and plateau potentials of most stomatogastric neurons result from the operation of the same types of voltage- and Ca^{2+}-dependent ionic channels that are present in cardiac ganglion neurons. Regardless, their functional roles bear striking similarities.

Driver potentials – how widespread are they?

The existence of a mechanism endowing an individual neuron with the capability of responding to a simple input with a sustained and patterned output

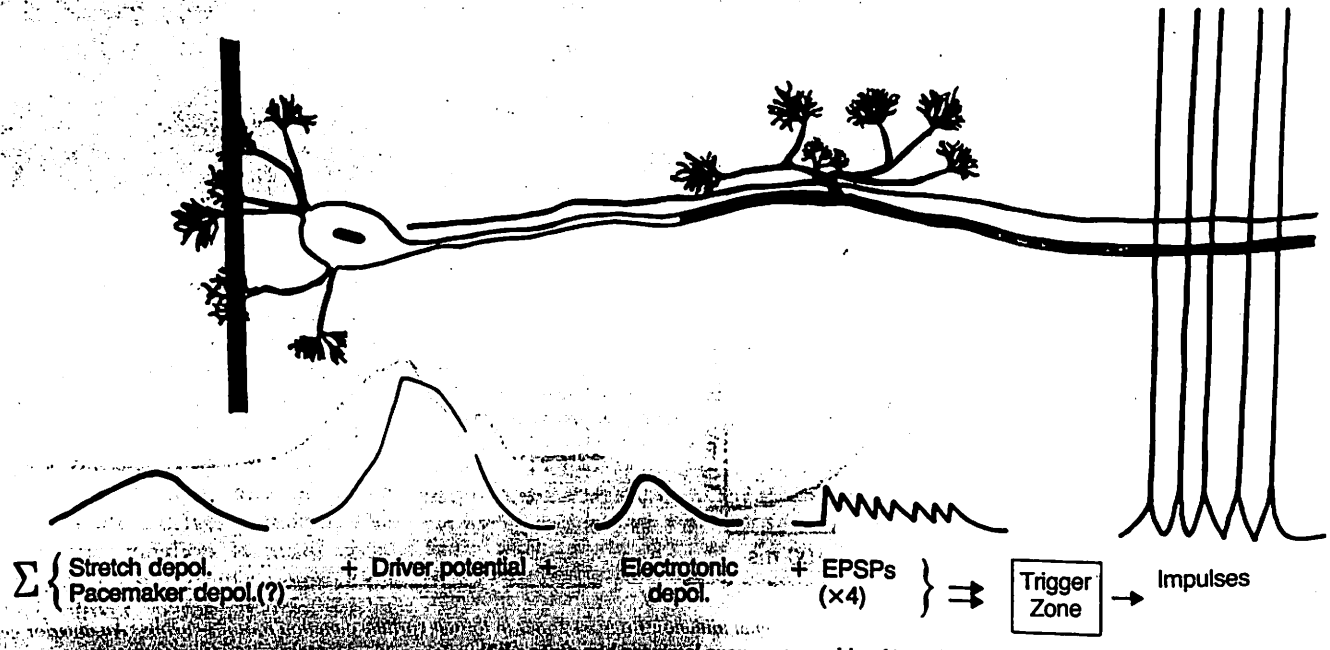

Fig. 6. Diagram depicting the supposed integrative region of the soma and proximal axon, not capable of impulse initiation, in which pacemaker depolarizations (if present), inputs of stretch-sensitive dendrites, and electrotonic and chemical synaptic inputs (on axon collaterals) all sum with the regenerative, non-propagating driver potential to produce a flow of current through the membrane of the more distal axonal trigger zone to initiate and influence the temporal patterning of the action potentials which propagate centrally.

(i.e. a train of temporally structured action potentials of a certain duration) potentially greatly simplifies the analysis of complex neuronal pattern generators. Only the timing of initiation of the burst-forming potential needs to be controlled. The utilization of the same group of neurons organized in a different pattern to produce a different behavior might be particularly facilitated by the ability to synaptically or hormonally control whether individual neurons will produce a burst.

So far, driver potentials have been identified in the cardiac and stomatogastric ganglia of Crustacea only. The slow potentials of stomatopod cardiac ganglion neurons have properties indicating that they are probably driver potentials[24]. However, in *Limulus*, a primitive arthropod, the motor neurons of the cardiac ganglion discharge bursts of action potentials surmounting a depolarized plateau[25], but experiments have so far failed to reveal an underlying driver potential (Augustine, G., unpublished observations).

The most extensively studied endogenously bursting neurons are those of the molluscs where a slowly depolarizing pacemaker potential leads to a long depolarization surmounted by overshooting action potentials. The burst-generating mechanism in molluscan endogenous bursters is confined to the soma and proximal axon, but action potential generation is an intrinsic and essential part of the burst cycle (Adams, W. B., unpublished observations). Despite the observation in molluscan bursters of square-shaped, slow depolarizations in unusual conditions following TTX treatment, it seems unlikely that driver potentials, as they have been defined for Crustacea, will be found to contribute to bursting activity in molluscs.

Among the vertebrates, vasopressinergic neurons cultured from fetal mice show unusually long (30 s) Ca^{2+}-dependent plateau potentials[26]. Purkinje cell dendrites show plateau potentials[27]. Bursting, with underlying slow depolarization, and dependence of both the slow depolarization and of bursting on the presence of Ca^{2+} in mammalian thalamic neurons, make these candidates for exhibiting driver potentials[28,29].

While driver or plateau potentials are not invariably associated with impulse bursting in neurons, they have been observed sufficiently frequently to suggest that they should be looked for when analysing pattern-generating neuronal networks. Certainly in arthropods they can play a major role not only in endowing individual neurons with the ability to produce patterned output to simple inputs, but also in mediating interneuronal co-ordination via electrotonic or non-impulse-mediated synaptic transmission.

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The importance of both early and delayed responses in the biological actions of nerve growth factor

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A general principle that has emerged from the study of intercellular signals is that these agents often promote both rapidly onsetting and delayed responses. For example, insulin enhances the uptake of glucose within minutes of its application to target cells. In contrast, other responses to insulin, such as stimulation of DNA synthesis, are apparent only after latencies of tens of hours. The object of this article is to review evidence that nerve growth factor (NGF), a protein with a variety of actions on vertebrate dorsal root sensory and sympathetic neurons[1–3], also triggers both rapid and delayed responses. In addition, we raise the issue that the highly asymmetric form of neurons as well as their trophic interactions have brought this duality of action to an especially, and perhaps uniquely, important role in the nervous system.

Experimental systems

A major aid in studying the multiple actions of NGF is that many of them can occur in cell or tisssue culture[1–3]. Two complementary *in vitro* systems have been widely employed in this regard – primary cultures of ganglionic sympathetic and sensory neurons, and established clonal lines of NGF-responsive tumor cells. Of the latter, the PC12 line of rat