Conditional dendritic oscillators in a lobster mechanoreceptor neurone

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1. Intra- and extracellular recordings were made from *in vitro* preparations of the lobster (*Homarus gammarus*) stomatogastric nervous system to study the nature and origin of pacemaker-like activity in a primary mechanoreceptor neurone, the anterior gastric receptor (AGR), whose two bilateral stretch-sensitive dendrites ramify in the tendon of powerstroke muscle GM1 of the gastric mill system.

2. Although the AGR is known to be autoactive, we report here that in 20% of our preparations, rather than autogenic tonic discharge, the receptor fired spontaneously in discrete bursts comprising three to ten action potentials and repeating at cycle frequencies of 0·5–2·5 Hz in the absence of mechanical stimulation. Intrasomatic recordings revealed that such rhythmic bursting was driven by slow oscillations in membrane potential, the frequency of which was voltage sensitive and dependent upon the level of stretch applied to the receptor terminals of the AGR.

3. Autoactive bursting of the AGR originated from an endogenous oscillatory mechanism in the sensory dendrites themselves, since (i) during both steady, repetitive firing and bursting, somatic and axonal impulses were always preceded 1:1 by dendritic action potentials, (ii) hyperpolarizing the AGR cell body to block triggering of axonal impulses revealed attenuated somatic spikes that continued to originate from the two peripheral dendrites, (iii) the timing of burst firing could be phase reset by brief electrical stimulation of either dendrite, and (iv) spontaneous bursting continued to be expressed by an AGR dendrite after physical isolation from the GM1 muscle and the stomatogastric nervous system.

4. Although a given AGR *in vitro* could switch spontaneously from dendritic bursting to tonic firing and vice versa, exogenous application of micromolar (or less) concentrations of the neuropeptide F1 (TNRNFLRFamide) to the dendritic membrane could rapidly and reversibly switch the receptor firing pattern from repetitive firing to the bursting mode. Exposure of the somatic and axonal membrane of the AGR to F1 was without effect, as were applications of other neuroactive substances such as serotonin, octopamine and proctolin.

5. We conclude that, as for many oscillatory neurones of the central nervous system, the intrinsic activity pattern of this peripheral sensory neurone may be dynamically conferred by extrinsic modulatory influences, presumably according to computational demands. Moreover, the ability of the AGR to behave as an endogenous burster imparts considerable integrative complexity since, in this activity mode, sensory coding not only occurs through the frequency modulation of on-going dendritic bursts but also via changes in the duration of individual bursts and their inherent spike frequencies.

It is now clear that many neurones of the central nervous systems of invertebrates and vertebrates alike are endowed with voltage-dependent membrane properties in addition to those directly responsible for the generation of action potentials. One notable type of such intrinsic neuronal behaviour is that responsible for membrane potential oscillation and rhythmic bursting. The list of neurones found to express this non-linear capability is now extensive, including cell types involved in invertebrate and vertebrate rhythmic motor systems (for reviews, see Selverston &

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† This paper is dedicated to the memory of Professor Maurice Moulins who died in December 1995.

Moulins, 1985; Grillner, Wallèn, Brodin & Lansner, 1991), neurosecretory processes in both molluscs (Benson & Adams, 1989) and mammals (Andrew, 1987), and a variety of other regulatory functions and pathological disorders of the vertebrate brain (Llinás, 1988).

From a number of studies, particularly with the advent of vertebrate slice preparations, it is also known that the capacity of an individual neurone to oscillate and fire in rhythmic bursts may be an intrinsic property of its dendritic membrane (Llinás, 1988). In this situation, therefore, the dendrites of these central neurones do not behave as passive conveyors of afferent information towards the soma and axon but, due to their own active membrane properties, are responsible for highly non-linear relations in the transformation of synaptic input into axonal output firing. Furthermore, despite the diversity in location and function of intrinsically bursting neurones, a feature common to many of these cells is that expression of their oscillatory capability is not invariant, but can be modulated by a variety of neuroactive substances (e.g. Dekin, Richerson & Getting, 1985; McCormick & Pape, 1990*b*; Ramirez & Pearson, 1991; Harris-Warrick, Nagy & Nusbaum, 1992). In this way, the presence of a modulatory transmitter or hormone may be able to specify different intrinsic activity patterns in a target neurone, including causing it to switch from repetitive firing to bursting modes, as integrative contingencies demand.

In contrast to the substantial documentation of endogenous burst-generating behaviour in central neurones, reports of such activity in the peripheral nervous system are relatively rare. Apart from extracellular evidence that mammalian thermoreceptors may act as peripheral burst pattern-generators whose frequency is temperature dependent (Iggo, 1969), until recently the only 'pacemaker-like' activity attributed to primary sensory neurones is the capacity for autogenic repetitive firing (Wiederhold & Carpenter, 1982). It is now apparent, however, that electrosensory neurones of fish express autoactivity (Turner, Maler, Deerinck, Levinson & Ellisman, 1994) which may take the form of bursting behaviour (Braun, Wissing, Schäfer & Hirsh, 1994), with potentially important consequences for sensory processing related to electrolocation. To date, however, direct evidence for an endogenous burst-generating capability in primary mechanoreceptor systems is sparse, the most persuasive data arising from studies on intrinsic resonance phenomena in the vertebrate vestibular system (e.g. Fuchs, Nagai & Evans, 1988; Sugihara & Furukawa, 1989) and muscle spindles of frogs (Sokabe *et al.* 1993).

In an earlier study we reported that a single mechanoreceptor neurone, the anterior gastric receptor (AGR) in the lobster stomatogastric nervous system possesses an autoactive tonic firing capability (Simmers & Moulins, 1988*a*; Combes, Simmers, Nonnotte & Moulins, 1993). Furthermore, we demonstrated that this intrinsic excitability is mechanosensitive (see also Combes, Simmers & Moulins, 1995*b*) and is probably mediated by spike-generating Na^+ conductances situated on the AGR stretch-sensitive dendrites themselves (Combes *et al.* 1993). Here we show that this primary mechanoreceptor neurone, like many central neurones, can oscillate and discharge in rhythmic bursts. These intrinsic membrane oscillations are mechanosensitive and are generated by dendritic membrane, close to the peripheral receptor terminals. Furthermore, we found that the neuropeptide TNRNFLRFamide (or F1 peptide), a member of the FRMFamide family first isolated from lobster (Trimmer, Kobierski & Kravitz, 1987) and known to modulate arthropod mechanoreceptors (Pasztor & Golas, 1993), reliably transformed the discharge pattern of tonically firing AGR into a rhythmic bursting mode. This conditional endogenous bursting mechanism considerably augments the encoding capacities of the receptor by providing an intrinsic rhythmic substrate that can be further modified by extrinsic mechanical input. A preliminary account of some of these data has been published (Combes, Simmers & Moulins, 1992).

METHODS

Experiments ($n = 40$) were performed on the anterior gastric receptor of 250–400 g adult European lobsters, *Homarus gammarus*, obtained commercially and maintained in fresh running seawater until used. General dissection procedures were as described previously (Simmers & Moulins, 1988*a*) for *in vitro* preparations of the stomatogastric nervous system. This comprised the stomatogastric ganglion, the stomatogastric nerve and short sections of the inferior oesophageal nerves (which normally carry the axon branches of the AGR to their corresponding commissural ganglia) and the bilateral branches of the anterior gastric nerves that contain the left and right dendritic processes of the AGR (Fig. 1*A*). In most experiments, the portion of the stomach wall that carries the insertions of the two bilateral bundles of gastric muscle GM1 was also dissected free and the receptor endings of the AGR in the muscle tendon (Combes *et al.* 1995*b*) were left intact. This allowed the receptor to be stimulated mechanically with trapezoidal or ramp stretches applied by an electromechanical puller attached to either of the two dorsal sclerites onto which the bilateral GM1 muscle bundles insert. In some experiments, the fibre bundles of one or both sides were cut short and the dendritic processes of the AGR, still attached to the stomatogastric nervous system, were dissected free and pinned out on the Sylgard®-lined Petri dish. Preparations were superfused continuously with oxygenated artificial seawater maintained at 15–18 °C with a laboratory-constructed thermoelectric cooling system and containing (mM): 400 Na^+, 9·8 K^+, 10·1 Ca^{2+}, 52·6 Mg^{2+}, 28 SO_4^{2-} and 535 Cl^-, buffered to pH 7·45 with 2·5 $NaHCO_3^-$.

Extracellular recording/stimulation of the AGR was performed with fine platinum wire electrodes isolated electrically with Vaseline after placement against the left and/or right dendritic branches and either inferior oesophageal nerve which carries the axon of the receptor. Intracellular recordings were also made from the AGR with a 3 M KCl-filled microelectrode (tip resistance, 10–20 MΩ) inserted into the bipolar soma of the receptor after desheathing the initial portion of the medial nerve immediately posterior to the stomatogastric ganglion. Single electrode recording and current injection were achieved via the bridge circuit of an M707 electrometer from World Prescision Instruments (Sarasota, FL, USA).

To examine the effects of serotonin, octopamine, proctolin (all purchased from Sigma) and the F1 neuropeptide (Cambridge Research Biochemicals, Northwich, Cheshire, UK) on the electrical behaviour of the AGR, various concentrations (less than 10^{-5} M) of these substances were added to the saline bathing the entire preparation, or were applied locally to the receptor endings via a Vaseline well built around one or both dendrites. In some cases, to block action potentials, 10^{-6} M tetrodotoxin (TTX) was applied focally to the axonal or dendritic membrane of the receptor via a manipulator-mounted micropipette containing the toxin and coupled to a Picospritzer (see Combes *et al.* 1993). Conventional techniques were used for display, storage and transcription of recorded data.

RESULTS

Anterior gastric receptor (AGR)

Simmers & Moulins (1988*a*) first reported a primary mechanosensory neurone, the AGR, associated with the bilateral bundles of the powerstroke, GM1, muscle of the gastric medial tooth in the lobster stomach. Previously

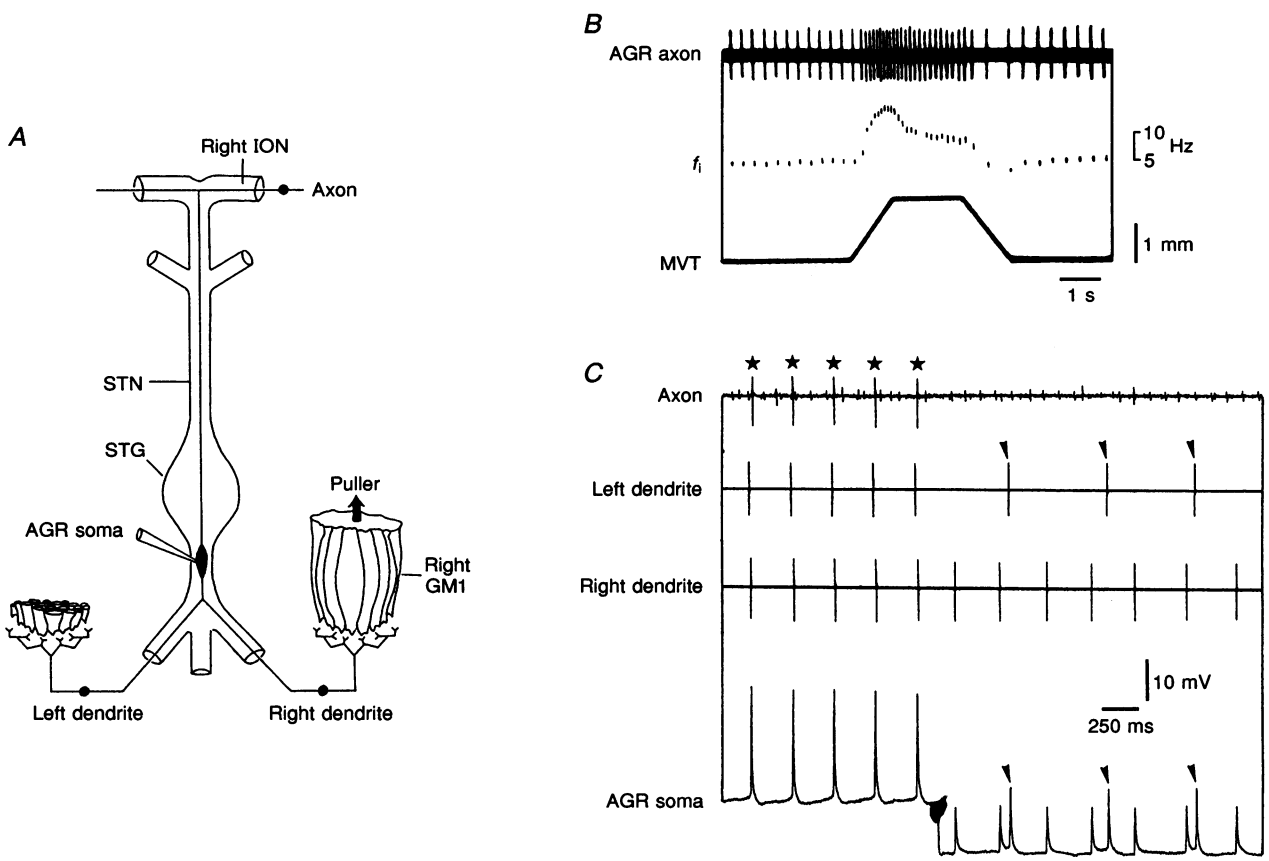


Figure 1. Location and physiology of the anterior gastric receptor (AGR) in the European lobster, *Homarus gammarus*

A, schema of stomatogastric nervous system–muscle preparation used to investigate spontaneous firing patterns of the AGR and its responsiveness to mechanical or electrical stimulation. The left and right gastric GM1 muscles along with the underlying stomach cuticle were left attached (via the bilateral nerve roots that carry AGR dendrites) to the isolated stomatogastric nervous system. In most preparations the bilateral muscles were shaved (as illustrated for the left-hand side), leaving the tendon and AGR dendritic terminals intact. In some preparations, one GM1 was left intact (see right-hand side) to allow a mechanical puller to be fixed to the dorsal sclerite onto which the muscle inserts. Sites of intracellular recording (from the soma of the AGR near the stomatogastric ganglion, STG) and extracellular recordings (●) are indicated. STN, stomatogastric nerve; ION, inferior oesophageal nerve. *B*, sensitivity of the AGR to mechanical stimulation. Axonal spike activity of the AGR recorded in the ION (see *A*) and corresponding instantaneous frequency transform (f_i) during a single ramp stretch of muscle GM1. Note the pronounced dynamic component of the AGR response superimposed upon autogenic tonic firing in the absence of stimulation. MVT, movement monitor. *C*, bilateral dendritic origin of autogenic firing of the AGR. Spontaneous receptor activity recorded simultaneously from the left and right dendrites and soma at resting potential (−65 mV) (left-hand side of panel) shows impulses occurring 1 : 1 with propagated spikes (indicated by ★) in the axon of the cell. Injection of hyperpolarizing (−1·5 nA) current prevents large amplitude soma potentials and axon spikes, and reveals smaller intrasomatic potentials preceded 1 : 1 by impulses occurring along the left (arrowheads) or right dendrite.

reported features of this unique receptor are summarized in Fig. 1. The two main dendrites of the AGR, which arise from the tendon of GM1 and respond to either passive muscle stretch (Fig. 1*B*) or active GM1 contraction (Combes *et al.* 1995*b*), converge medially onto a single apical dendrite that enters a bipolar cell body located posterior to the stomatogastric ganglion (Fig. 1*A*). The axon of the receptor runs in an anterior direction through the stomatogastric ganglion and into the stomatogastric nerve, which normally projects via the left and right inferior oesophageal nerves to the bilateral commissural ganglia (not shown in Fig. 1*A*). In each commissural ganglion, the AGR excites two identified interneurones (Simmers & Moulins, 1988*a*,*b*; Combes, Simmers, Meyrand & Moulins, 1995*a*), which in turn descend to the stomatogastric ganglion in the stomatogastric nerve to influence motoneurones innervating GM1 and other gastric mill muscles.

Autogenic dendritic firing

A feature of the AGR, both in isolated stomatogastric nerves lacking any gastric musculature and in minimally dissected preparations, is that the cell is always spontaneously active (Simmers & Moulins, 1988*a*; Combes *et al.* 1993). As seen on the left-hand side of Fig. 1*C*, this autoactivity typically consists of regularly spaced action potentials occurring at 3–15 Hz in the axon of the cell, soma and two main dendrites. The dendritic origin of this autogenic firing can be demonstrated by using intrasomatic hyperpolarizing current injection (Fig. 1*C*, right-hand side) to block axonal spike generation and reveal smaller depolarizing transients in the soma, which follow impulses 1:1 occurring along one or other of the main dendrites of the receptor. These potentials arriving from the two sides can be distinguished in the intrasomatic recording on the basis of their different amplitudes (for example, the arrowheads in Fig. 1*C* indicate impulses in the left dendrite and their corresponding potentials in the soma). In effect, as proposed by Combes *et al.* (1993), a direct dendro-dendritic interaction normally co-ordinates these bilateral impulses, which in turn traverse the single apical neurite to eventually activate a third spike-initiating zone on the initial axon segment (as in Fig. 1*C*, left-hand side). With sufficient experimental soma hyperpolarization (as in Fig. 1*C*, right-hand side), the spread of injected current away from the soma is able to prevent axonal impulses and block the bilateral dendritic interaction, presumably by altering the safety factor for transmission through the common branch point with the apical neurite. Under these conditions, the

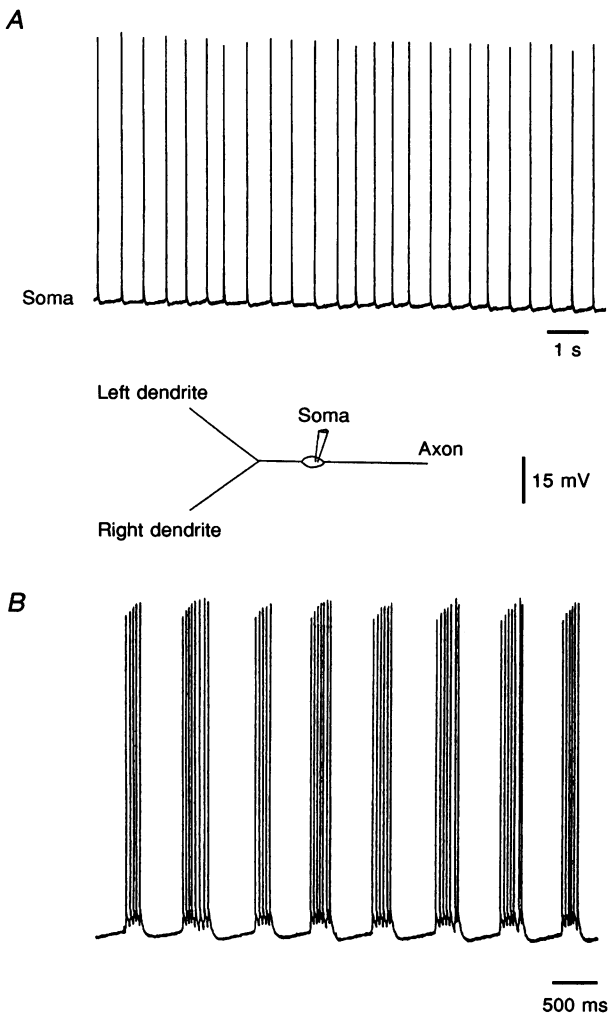


Figure 2. The AGR can express two types of autogenic activity

A, intrasomatic recording (see schema) of an AGR showing typical (80% of preparations) spontaneous tonic firing. *B*, a different cell expressing spontaneous activity seen in the remaining *in vitro* preparations; rhythmic bursts of impulses atop smooth membrane potential oscillations. Most hyperpolarized membrane potentials: *A*, −61 mV; *B*, −62 mV.

separate spike trigger zones of the two dendrites become functionally decoupled, allowing them to fire at their different inherent frequencies.

Endogenous bursting

In contrast to the spontaneous tonic firing seen in 80% of AGR recordings *in vitro* (Fig. 2*A*; see also Fig. 1*B* and *C*), in a minority of isolated stomatogastric nerve preparations ($n = 8$) the receptor exhibited a different type of auto-activity that consisted of discrete bursts of action potentials (Fig. 2*B*). This phasic discharge comprised trains of between three and ten impulses in different preparations, repeating at cycle frequencies of 0·5–2·5 Hz and riding atop smooth 2–10 mV oscillations in membrane potential. Although discrete synaptic potentials have never been observed in our intracellular recordings from the AGR soma, it is conceivable that this rhythmicity is driven by a synaptic input that somehow overrides the tonic intrinsic excitability of the receptor and forces the cell to fire in phasic bursts. However, several lines of evidence suggest that this is not the case. Firstly, consistent with the lack of evidence for postsynaptic potentials in our physiological recordings (see also Combes *et al.* 1993), no morphological evidence has been found for any synaptic contacts onto AGR soma–dendritic regions (Combes *et al.* 1995*b*). Secondly, the oscillatory activity that underlies AGR bursting expresses major features of an intrinsically driven mechanism. These include a membrane potential trajectory strongly reminiscent of classical pacemakers (Benson & Adams, 1989), displaying a slow ramp-like depolarization which eventually leads to a rapidly rising and falling depolarizing hump that drives each spike burst (Fig. 2*B*, see also Fig. 4*A*). Consistent with an endogenous origin (Frazier, Kandel, Kupfermann, Waziri & Goggeshall, 1967), moreover, the frequency of oscillation and bursting is strictly dependent upon the cell membrane potential. This can be seen in Fig. 3*A*, where continuous intrasomatic depolarization with injected current (Fig. 3*A a*, top two traces) increased the burst frequency of an AGR (from a mean ± S.D. of 2·1 ± 0·1 Hz without current to 4·6 ± 0·3 and 8·8 ± 0·3 Hz with +1·0 and +2·5 nA, respectively), whereas steady hyperpolarization (−0·5 nA, Fig. 3*A a*, bottom trace) decreased the frequency (to 0·7 ± 0·1 Hz). As shown in Fig. 3*A b*, the receptor burst frequency varied almost linearly with such current-induced modifications in membrane potential, accelerating steadily to *ca* 11 Hz with depolarizations of +3·6 nA, while slowing and ceasing altogether with hyperpolarizations greater than −1 nA. Unlike cycle frequency, however, the duration of individual bursts and their spike numbers (3 per burst in this case) were relatively insensitive to experimental changes in membrane potential (compare traces in Fig. 3*A a*).

Also, like endogenous oscillators in general (Pinsker, 1977), the phase of on-going rhythm of the AGR could be reset by pulsed experimental perturbations. This is illustrated in Fig. 3*B*, where release from a brief (50 ms) intracellular injection of hyperpolarizing current (−1·5 nA) during spontaneous oscillations produced a premature burst (by rebound excitation) when delivered relatively early in the interburst interval (top trace, Fig. 3*B a*) and consequently caused a phase advance in the timing of subsequent cycles. By contrast, a similar negative pulse delivered later in the interburst interval (bottom trace, Fig. 3*B a*) retarded the onset of the next spontaneous burst and caused subsequent bursts to be phase delayed. As seen in the corresponding phase–response curve (Fig. 3*B b*), the extent of this resetting depended strictly on the phase of the burst cycle at which the stimulus was given, varying monotonically between maximal phase shifts (approaching 0·5) at the beginning (phase advance) and end (phase delay) of each cycle. In addition to intrasomatic current injection, brief extracellular stimulation of either of the AGR main distal dendrites could also stably reset the pacemaker cycle of the cell. This is illustrated in Fig. 3*C*, where a single brief shock (3 V, 1·2 ms) to a dendrite could trigger an earlier oscillation when delivered in the interburst interval, after the refractory period of the preceding spontaneous oscillation. The resulting phase advance in the timing of subsequent cycles again suggests that the perturbation has direct access to the oscillator mechanism. Similar continuous and pulsed current-induced modifications in on-going rhythmicity to those seen in Fig. 3 were observed in all five spontaneously bursting preparations tested. From these observations, therefore, we conclude that autogenic burst generation in the AGR arises from an intrinsic membrane property of the receptor itself.

A dendritic oscillator

We were next interested in determining the site at which these intrinsic oscillations originated. Are they generated near the soma–axon boundary, along the apical neurite or even more distally along the bilateral dendritic arbors of the cell? This question was addressed in the experiments illustrated in Figs 4 and 5, where again advantage was taken of the accessibility and large geometry of the AGR to make simultaneous recordings from different cellular compartments during spontaneous bursting activity. Firstly, as seen in Fig. 4*A* ($n = 7$), impulses throughout each burst recorded on the dendrite of the receptor always preceded those recorded in the soma and, in turn, the cell axon in the stomatogastric nerve (see Fig. 4*A b*). Secondly, dendritic oscillation and bursting could be expressed in the absence of axonal action potentials. This is evident in Fig. 4*B*, where TTX was focally applied (see Methods) to the initial axon segment to block fast sodium channels in this region. In the presence of the toxin ($n = 4$), the large depolarizing transients (corresponding to spikes generated on the nearby axon hillock) were seen to gradually drop out in the soma recording, revealing uninterrupted oscillatory and decremented bursting activity associated with extracellularly recorded impulses in a remaining receptor dendrite. By contrast, after washout in control saline, TTX now selectively applied to the dendrite rather than the axon rapidly and completely suppressed all spontaneous rhythmic activity (data not shown). It is noteworthy also that the

spontaneous bursting capability of the AGR, although slowed somewhat, was otherwise unaffected by replacing Ca^{2+} in the bathing solution with equimolar Mg^{2+}, or replacing Ca^{2+} with 10 mM Co^{2+} ions ($n = 2$). This is consistent with an oscillatory mechanism which, like the impulses it drives (Combes *et al.* 1993), involves Na^{+} rather than Ca^{2+} or Ca^{2+}-dependent conductances.

While these observations point strongly to the dendritic origin of AGR bursting activity, it remains possible that the

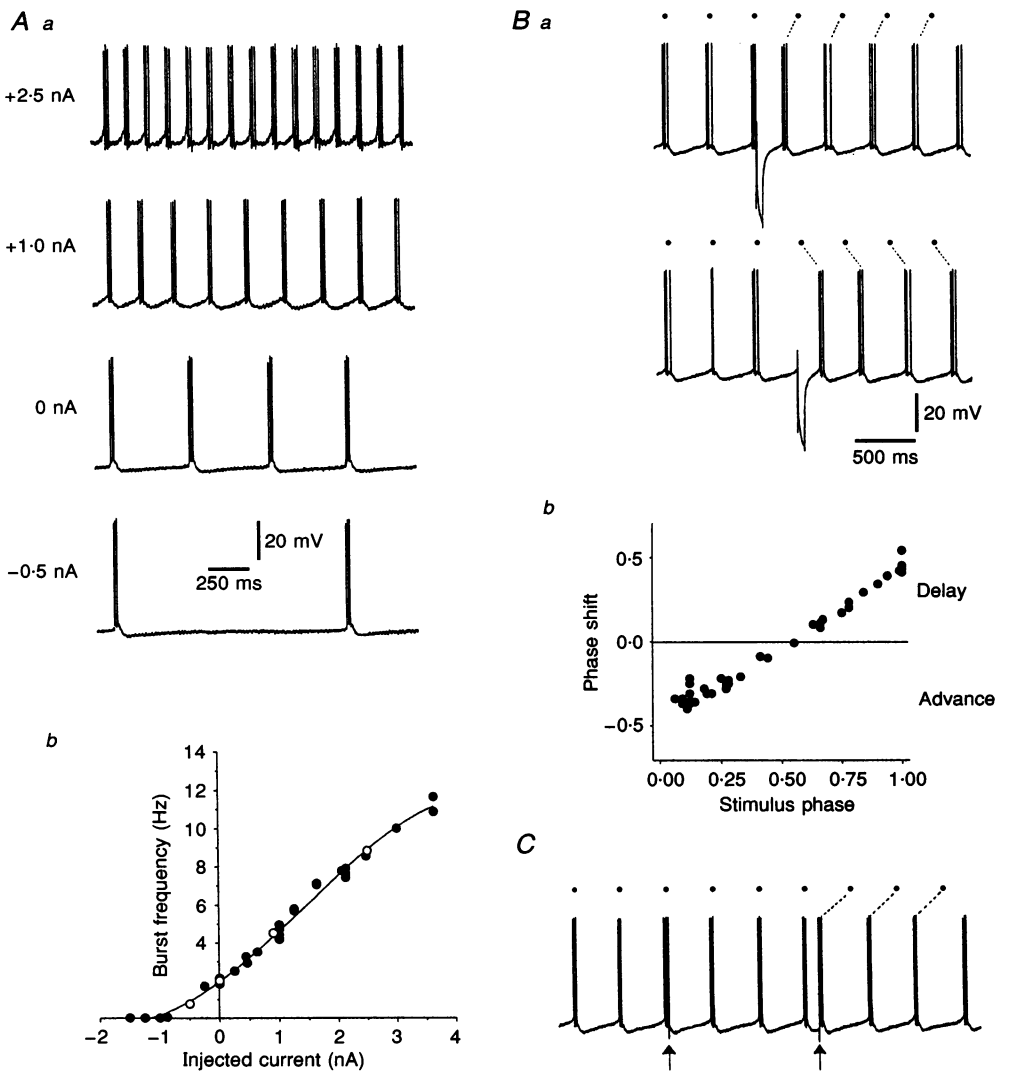


Figure 3. AGR bursting is an intrinsic membrane property

A, voltage dependence of oscillation frequency. *a*, intrasomatic recording of a spontaneously bursting AGR. Injection of constant depolarizing (+1·0 nA, +2·5 nA) or hyperpolarizing (−0·5 nA) current through the recording electrode causes burst frequency to increase or decrease, respectively. *b*, frequency of bursts as a function of the amount of injected current. Open circles correspond to the 4 traces illustrated in *a*. (Although absolute membrane potential was not plotted because of uncertainty of bridge circuit balance, V_m changes were *ca* 20 mV per nA injected.) Most hyperpolarized membrane potential in the absence of current injection, −61 mV. *B*, phase resetting of the oscillator cycle of the same receptor. *a*, brief hyperpolarizing pulses (−1·5 nA, 50 ms) injected into the soma causes the on-going cycle to be phase-advanced (upper trace) or phase-delayed (lower trace) depending on the timing of stimulation. Dots above each record indicate the expected timing of bursts in the absence of perturbation. *b*, plot of the phase shift of AGR oscillations as a function of the timing of stimulus delivery. Cycle periods and phases were calculated using at least 5 cycles before the perturbing pulse. *C*, a brief extracellular shock (3 V, 1·2 ms) applied to a dendrite of the same receptor can also reset the on-going rhythm when delivered in the interval between bursts. The same stimulus had no effect when applied immediately after a spontaneous burst (left-hand arrow), but caused a permanent phase advance when applied later in the interburst interval (right-hand arrow).

oscillatory mechanism is located at a communal site along the apical neurite, the region between the dendritic branches and the bipolar soma. However, in agreement with previous findings that the apical neurite of the AGR is probably inexcitable (Combes *et al.* 1993), there is direct evidence that burst generation arises more distally, from spatially separate oscillators on each of the two dendrites. The first direct indication for this distal location is evident from the experiment of Fig. 5*A* ($n = 4$), where injection into the soma of continuous hyperpolarizing current was used to block axonal spikes in a strongly bursting AGR in which both main dendrites remained attached. Under these conditions, and in a manner similar to that seen in Figs 1*C* and 4*B*, smaller depolarizing potentials due to continued dendritic activity were now evident. Significantly, inspection of this residual activity recorded in the soma shows two distinct populations of depolarizing transients with different amplitudes and grouped in bursts with different cycle periods. During tonic repetitive firing (Fig. 1*C*), this bimodal bursting activity is inferred to be driven by spontaneous input from each of the two dendritic branches (see d_1 and d_2 in Fig. 5*A*, right-hand side) that are normally

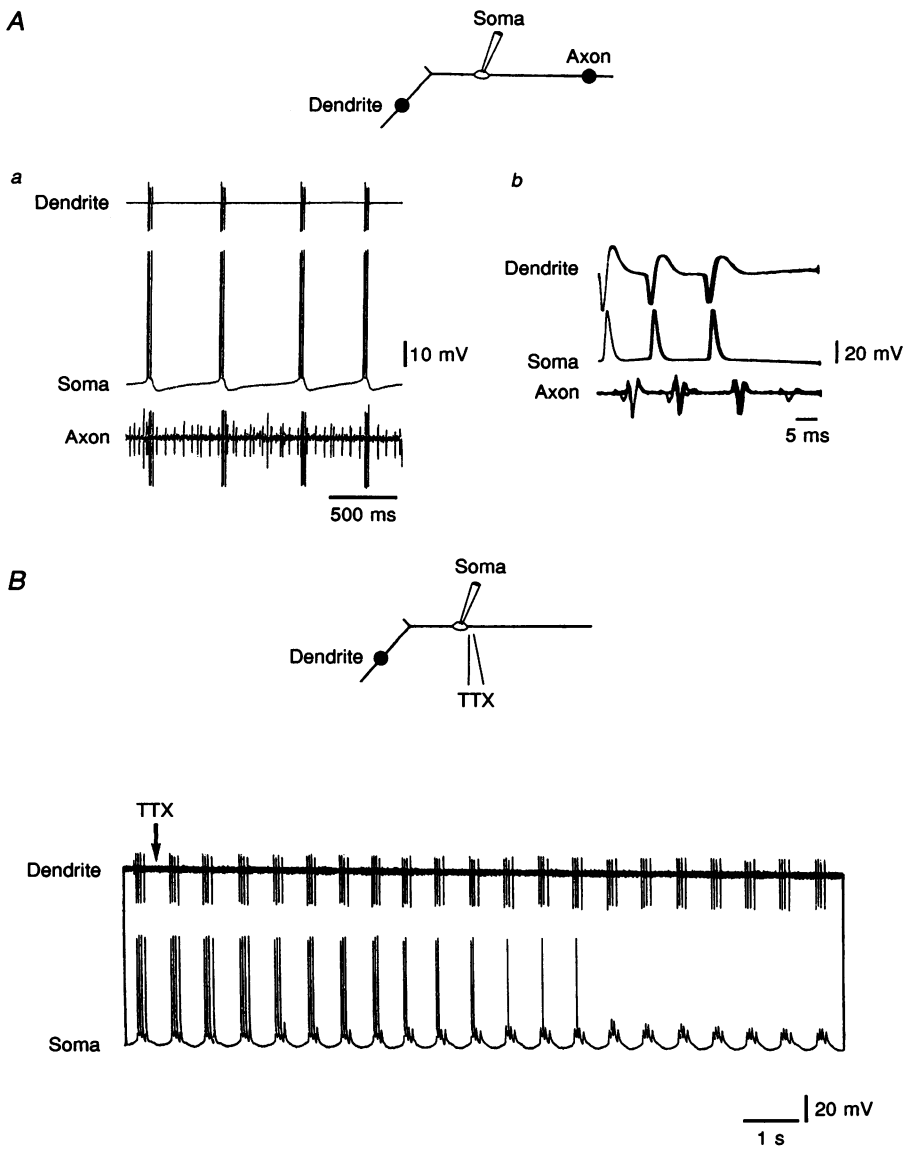


Figure 4. The oscillator is located in AGR dendrites

A, simultaneous recording from the soma, axon and a dendrite of the AGR, as in the schema. *a*, strict 1 : 1 correlation between bursting in the 3 cell regions. Note the slower, pacemaker-like potential underlying each spike burst in the soma record. *b*, 5 superimposed oscilloscope sweeps on a faster time base from a different AGR showing that dendritic action potentials precede somatic and axonal spikes in each burst. *B*, microapplication of TTX (10^{-6} M) to the soma–axon boundary (see schema) of a different AGR gradually blocks large amplitude axonal impulses, but the dendrite of the receptor continues to produce bursts of action potentials (upper trace) that can be seen in the soma (lower trace) as decremented transients on membrane potential oscillations. Most hyperpolarized membrane potential: *A*, −61 mV; *B*, −58 mV.

co-ordinated (as in Fig. 5*A*, left-hand side) but which are now free to operate at their different inherent levels of excitability. This independent autogenic ability is further confirmed in the experiment of Fig. 5*B* ($n = 3$), where spontaneous bursting continued to be expressed by an extracellularly recorded dendritic branch after physical disconnection from the remainder of the cell. We conclude, therefore, that this mechanoreceptor can behave as a true endogenous oscillator, capable of spontaneous repetitive firing or rhythmic bursts of action potentials, and that the oscillatory mechanism for both autoactivity patterns is located peripherally, in the membrane of the two main dendrites of the receptor.

A conditional oscillator

As mentioned above, expression of autoactive bursting is not a constant feature of the AGR, but was found to occur spontaneously in only 20% of our *in vitro* preparations. In two of these preparations, moreover, the receptor was seen to switch spontaneously from a preferred tonic autoactive mode to rhythmic bursting and vice versa during the course of the experiment. However, the transition between firing patterns is not simply dependent upon changes in membrane potential, as in thalamocortical neurones for example (McCormick & Pape, 1990*a*). Steady current injection (with at least ±3 nA to evoke polarizations of 40 mV or more) into tonically active receptors never elicited burst firing ($n = 28$; see Simmers & Moulins, 1988*b*), nor did similar membrane polarizations of an already bursting AGR (all 6 preparations tested) cause it to fire tonically (see Fig. 3*A*).

The expression of two distinct modes of autogenic behaviour by the AGR suggested that the switch from tonic firing to intrinsic bursting may be governed by extrinsic modulatory

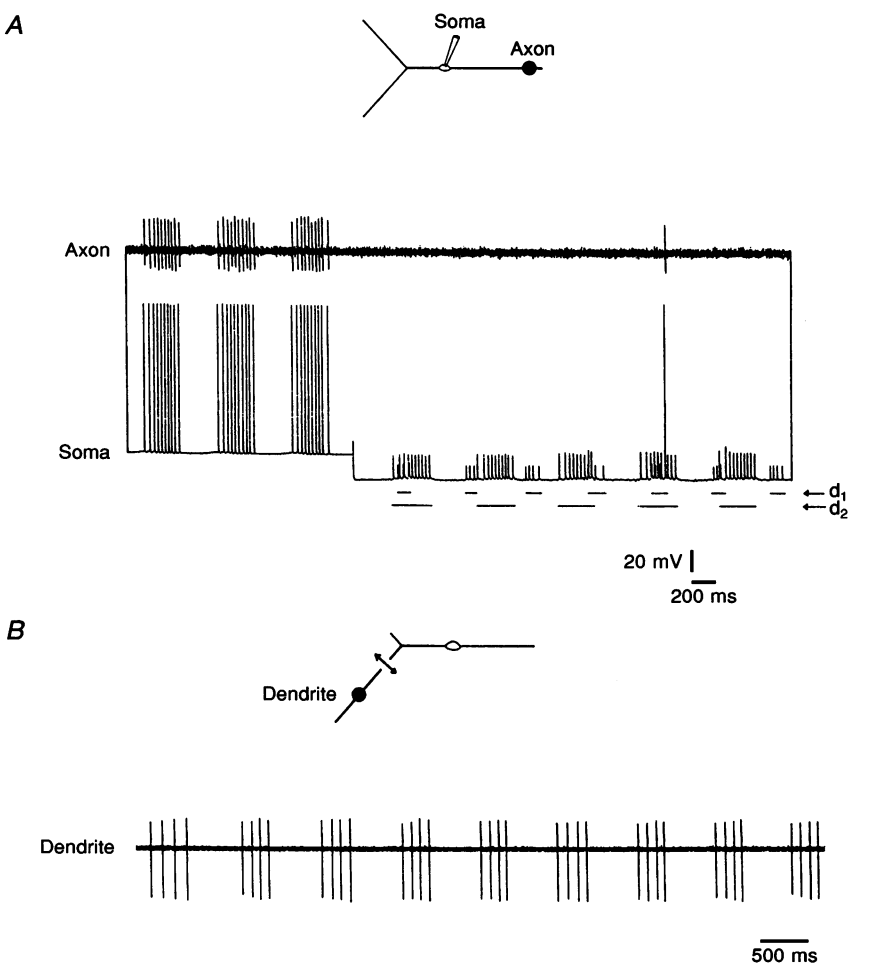


Figure 5. Both AGR dendrites possess an endogenous oscillator

A, simultaneous intra- and extracellular recordings from an AGR soma and axon, respectively, showing rhythmic axonal bursts (mean frequency, 1·6 Hz) that can be blocked by intrasomatic hyperpolarizing current injection (−1 nA). Under these conditions, remnants of impulse bursts arising from the two can be distinguished on the basis of differing spike amplitudes (d_1 and d_2 bursts, denoted by horizontal bars). Note that the two dendritic oscillators now free-run at their different inherent frequencies (d_1, 1·8 Hz; d_2, 1·4 Hz). Membrane potential in the absence of injected current: −61 mV. *B*, a single extracellularly recorded dendrite continues to generate rhythmic bursting after physical isolation from the rest of the neurone.

regulation, as in many endogenously active neurones of the central nervous system (see Discussion). To test this possibility, we investigated the influence on AGR intrinsic behaviour of a variety of neuroactive substances known to be present in neurones of the crustacean stomatogastric nervous system and/or to act as circulating hormones (see Harris-Warrick *et al.* 1992). In particular, attention was focused on four substances: the biogenic amines octopamine and serotonin, and the peptides proctolin and F1, all of which have been found to induce burst-generating properties in other stomatogastric neurones. The actions of these modulators were therefore assessed by perfusing them locally across a selected region of the AGR while recording from the AGR soma, axon or dendrite.

While both amines and proctolin (at concentrations up to 10^{-3} M) had no apparent influence on AGR autoactivity ($n = 4$ for each substance), application of exogenous F1 peptide was consistently found to alter the spontaneous firing pattern of the receptor reversibly from tonic to a bursting mode ($n = 11$ out of 14 preparations). In the experiment of Fig. 6, for example, before application of F1 (Fig. 6*A*), hyperpolarizing the receptor with injected current unmasked tonic dendritic impulses that were otherwise co-ordinated and generated axonal action potentials at around 6 Hz. In a first step, perfusion of 10^{-6} M F1 over the region of the soma and initial axon segment of the receptor had no effect on this on-going electrical activity of the cell (not shown). However, when the neuropeptide at the same

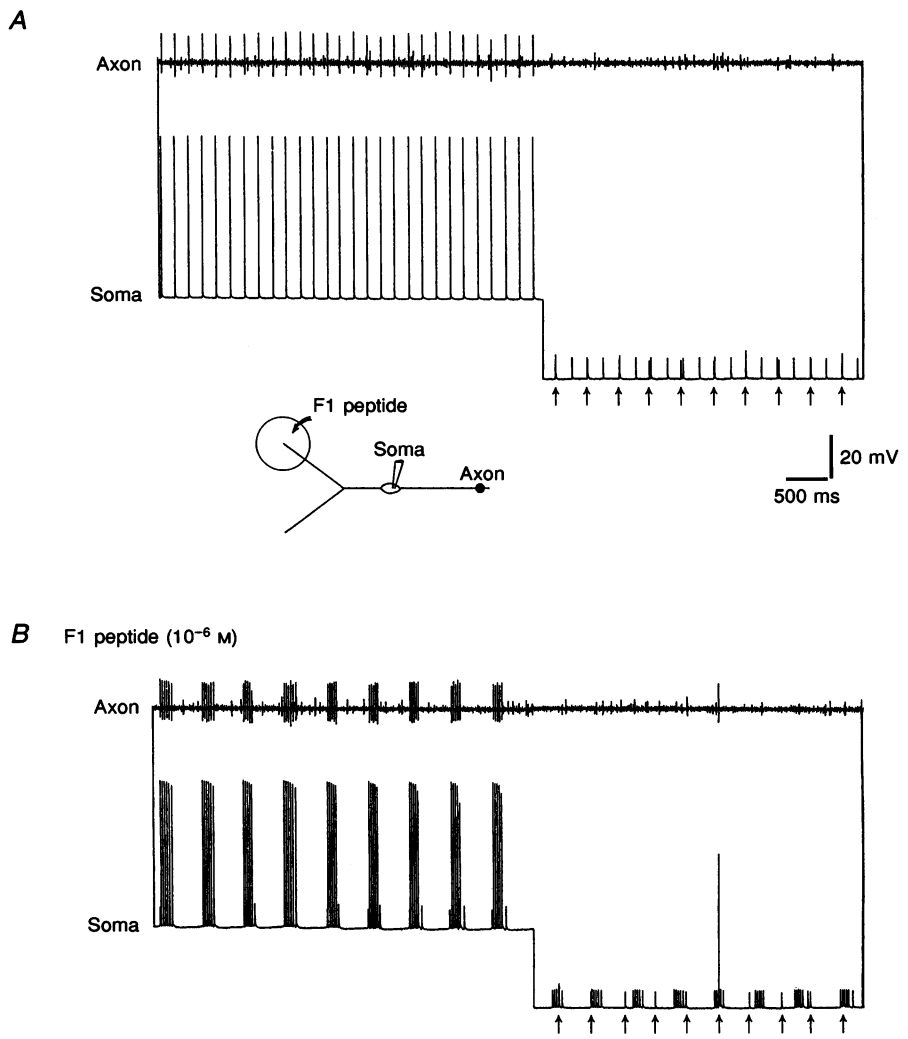


Figure 6. Exogenous F1 peptide can alter AGR firing pattern from tonic spiking to bursting

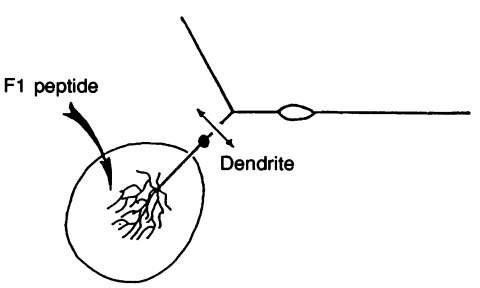
A, simultaneous intrasomatic and axonal recording from an AGR expressing spontaneous tonic firing. Soma hyperpolarization with injected current (−1·5 nA) prevents large amplitude spikes that propagate in the receptor axon, and reveals small amplitude dendritic potentials. *B*, in the same preparation, local application of F1 peptide (10^{-6} M) to one AGR dendrite (see schema) converts this tonic firing into bursting discharge. Hyperpolarizing current injection (−1·5 nA) to below axonal spike threshold now shows rhythmic bursts of potentials originating from the F1-treated dendrite, whereas impulses (arrowed) arising from the untreated contralateral dendrite continue to occur tonically at the same frequency as before (see *A*). Most hyperpolarized membrane potential in absence of injected current : *A*, −62 mV; *B*, −59 mV.

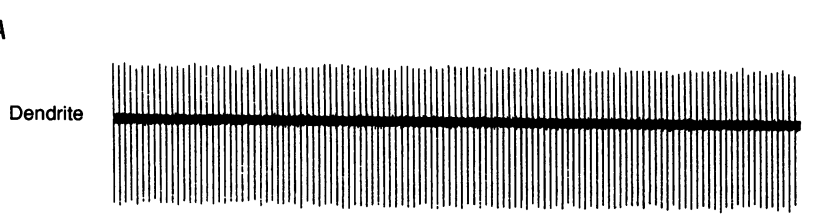
concentration was applied focally to a distal region of one of the AGR dendrites (see diagram in Fig. 6), the same cell began to fire rhythmically recurring bursts of five to eight impulses at a cycle period of around 500 ms (Fig. 6*B*). When soma hyperpolarization was now used to block axonal impulses and reveal activity of dendritic origin, this somatic activity could be differentiated into bursts of small potentials originating from the left dendrite that was exposed to exogenous F1. In contrast, the contralateral dendrite, which remained under normal saline, continued to fire tonically, as in control conditions (compare Fig. 6*A* and *B*; arrows indicate soma potentials arising from the right dendrite). This transition from tonic to burst firing in a single dendrite occurred within 10 s of the onset of perfusion with F1 and reversed over a time course lasting several minutes as the peptide was washed from the preparation.

That the switching action of peptide F1 is targeted at AGR peripheral dendrites is confirmed in the experiment of Fig. 7, where a completely isolated dendrite was recorded with an extracellular electrode before (*A*) and after (*B*) superfusion with the neuropeptide at 10^{-6} M. Here again, tonic autoactivity was reversibly transformed into robust bursting in the disconnected dendrite. Such switching effects of the F1 peptide were observed in experiments on both isolated dendrite ($n = 3$) and whole-cell preparations ($n = 8$). While most of these were performed with the peptide at 10^{-6} M, which was found to be the most effective concentration, similar actions were observed with concentrations down to 10^{-8} M. Although the effect of F1 on dendritic behaviour is fully reversible with washout, a single bath application of the peptide was found to strongly desensitize the dendritic membrane, so that a second application of F1 could completely fail to elicit bursting, even after extensive washing with control saline. Owing to this potent desensitization to exogenous F1, which has also been observed in peripheral nerve and muscle of lobsters and crayfish (Worden, Kravitz & Goy, 1995), it has not been possible to establish dose–response relationships for the action of the peptide on a single AGR.

Intrinsic oscillations and sensory coding

We were next interested in examining the relationship between different patterns of autogenic dendritic activity of the AGR and the role of the cell in mechanosensory processing. How is the intrinsic oscillatory mechanism of the receptor influenced by extrinsic mechanical input and what could be the functional consequences for sensory encoding of a capacity to switch between tonic and burst firing? To address these questions we used exogenous F1 peptide as a means of controlling the mode of autoactivity expressed by a given receptor, while applying mechanical stimulation to its still intact terminals in the tendon of the GM1 muscle (see Fig. 1*A* and diagram in Fig. 8). One such





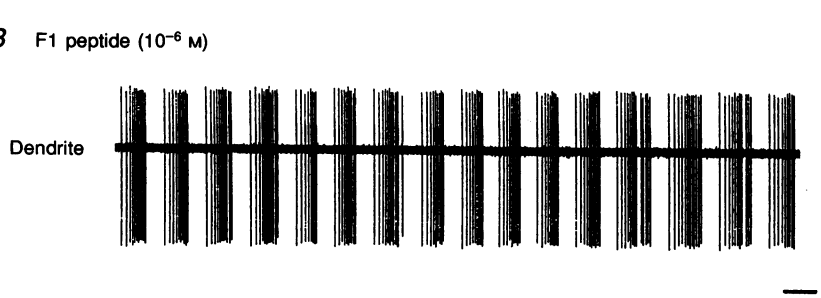


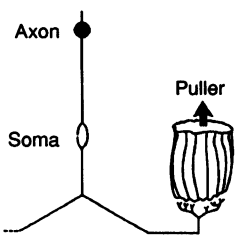
Figure 7. Peripheral action of the F1 peptide

Extracellular recording of an isolated AGR dendrite (see schema) before (*A*) and after (*B*) its autogenic firing was switched from tonic to rhythmic burst firing by superfusion with 10^{-6} M F1 peptide.

neuromuscular experiment is illustrated in Fig. 8, where trapezoidal ramp stretches were applied to a GM1 muscle while recording from the axon of the receptor in the stomatogastric nerve. In the absence of mechanical stimulation, this AGR expressed autogenic tonic firing at 3–4 Hz. When a ramp stretch of 1 mm in amplitude was applied to the GM1 muscle, the firing frequency of the receptor increased markedly, with a response envelope that showed an initial dynamic component (peaking at 14 Hz) during the movement, followed by a deceleration to a static discharge level (*ca* 8 Hz) that was maintained throughout the rest of the holding phase of the stimulus. As shown previously (Combes *et al.* 1995*b*), both dynamic and static components of the AGR firing rate changes increase with the velocity and amplitude of applied stretch, allowing the receptor to sense the phasic and static components of any tensile forces exerted on the tendon of muscle GM1.

When the tonically autoactive receptor in Fig. 8*A* switched to a bursting mode during exposure to 10^{-6} M F1 peptide (Fig. 8*B*), encoding of muscle stretch now became considerably more complex. In the presence of F1, but without mechanical stimulation, the receptor discharged in rhythmic bursts at around 1 Hz and comprising four to six impulses per burst. When a similar ramp stretch as before (cf. Fig. 8*A*) was now applied to the GM1 muscle, modifications were clearly evident in three different parameters of this bursting activity, namely an increase in the frequency of on-going bursts, an increase in spike rate within bursts and a decrease in their duration. In response to the 1 mm stretch in Fig. 8*B*, for example, AGR burst frequency increased from 1 Hz to more than 3 Hz, the mean spike frequency within bursts increased from 28 to 65 Hz, while burst duration decreased from an average of 400 ms to around 100–200 ms. It is noteworthy here that no observable differences were found between such mechanically evoked responses of an AGR in which bursting had been induced with exogenous F1 (as in Fig. 8*B*) and those observed in occasional neuromuscular preparations where the receptor was already bursting spontaneously.

In order to quantify the mechanosensitivity of AGR bursting behaviour, we measured burst frequency, spike frequency and burst duration during the course of repeated ramp stretches of constant amplitude applied to muscle GM1. One of five such experiments is illustrated in the three scatter diagrams of Fig. 9*A*, where instantaneous values for each parameter are plotted against time throughout twelve consecutive muscle extensions at an amplitude of 0·8 mm. The temporal relationship between each phase of the movement (schematized below Fig. 9*A*) is indicated by vertical lines. For all three parameters, the response peaked at the end of the initial dynamic phase of the movement, then tailed off during the subsequent static phase of the stimulus. For burst frequency (Fig. 9*A a*), a low plateau level is still evident at the end of the holding phase, with a



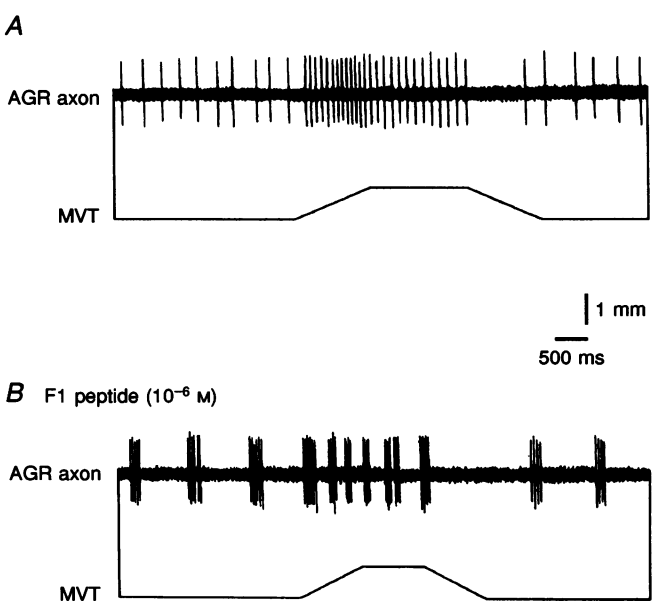


Figure 8. Comparison of AGR mechanosensitivity during tonic firing (*A*) and bursting (*B*) modes of activity

A, increase in discharge rate of a tonically autoactive AGR (recorded from its axon in the stomatogastric nerve; see schema) in response to a passive ramp stretch of the corresponding GM1 muscle (cf. Fig. 1*B*). *B*, same preparation after induction of receptor bursting with exogenous F1 peptide. A ramp stimulus now evokes an increase in the intensity and frequency of the otherwise spontaneously occurring bursts. Note the pronounced 'off' response at the end of each stimulation in *A* and *B*. MVT, movement monitor.

transient reduction in burst rate on release. In all three cases, there was no evidence of adaptation to repeated stimulation.

The three components of an AGR bursting response to mechanical stimulation varied with velocity and amplitude of muscle stretch (Fig. 9*B*). In this typical example, with GM1 relaxed, the receptor produced bursts at *ca* 1 Hz at rest, but with muscle extensions up to about 0·9 mm, the burst rate increased smoothly to nearly 2·5 Hz. Concomitantly, the spike frequency within these bursts climbed from a mean of 30 Hz to 40 Hz with stretches beyond 0·5 mm, while the duration of bursts declined from 270 to 200 ms. All three parameters continued to grade smoothly in a stimulus-dependent manner throughout stretches up to around 3 mm (not shown), which corresponds to the estimated maximum movement of the GM1 muscle *in vivo*. Thus, in contrast to a tonically active AGR where mechanical input is translated uniquely into different rates of repetitive firing (Fig. 8*A*), when the receptor is bursting (Figs 8*B* and 9), sensory encoding is now expressed in the frequency modulation of on-going dendritic bursts, and the duration of individual bursts as well as their internal spike frequencies.

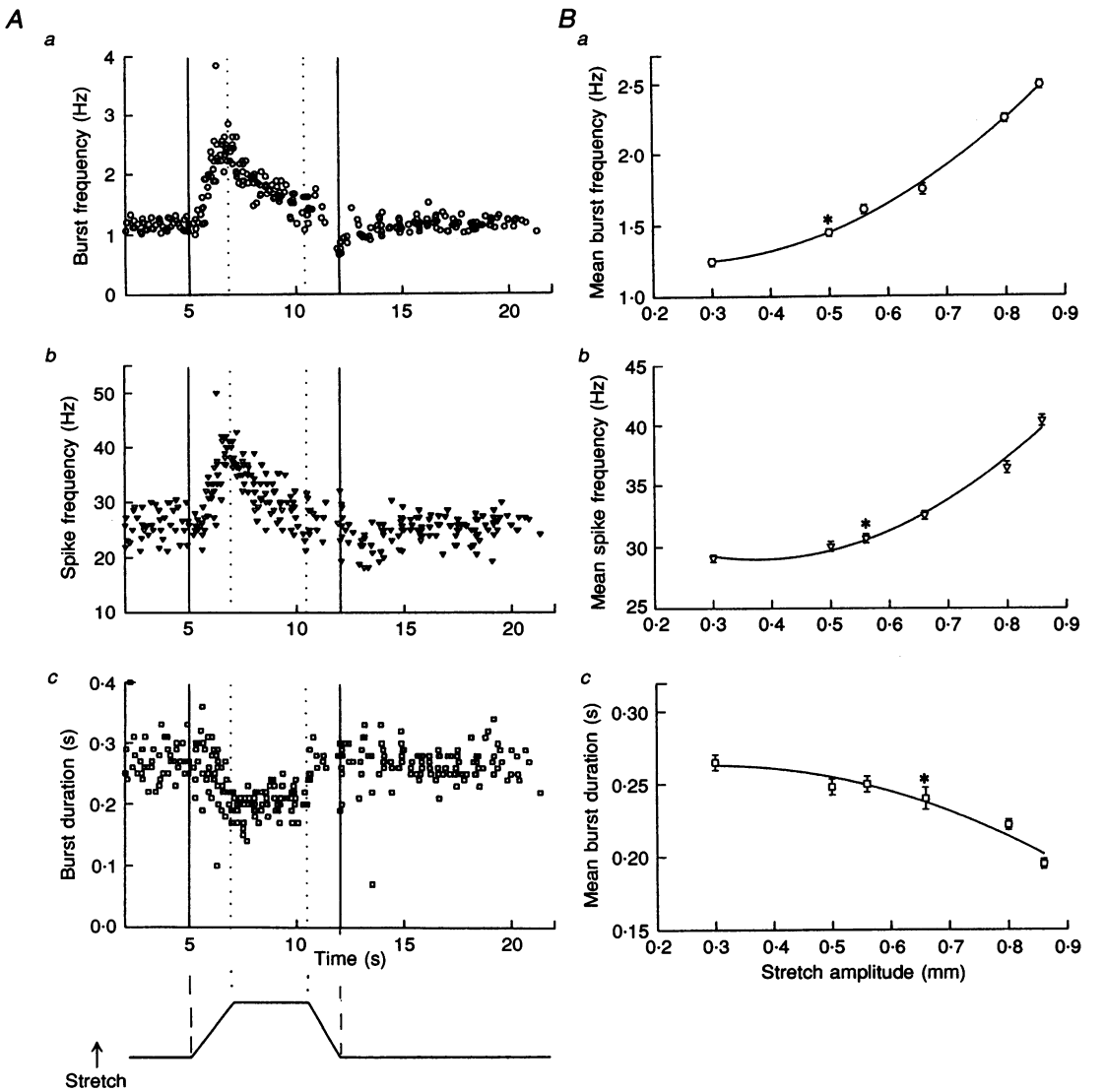


Figure 9. Multi-parametric encoding of mechanical stimulation by a bursting AGR

A, superimposed plots of instantaneous AGR burst frequency (*a*), spike frequency within bursts (*b*) and duration of bursts (*c*) during 12 consecutive and identical trapezoidal ramp stretches (amplitude, 0·8 mm) applied to a GM1 muscle. Vertical continuous lines indicate the beginning and end of stretches, while the dotted lines demark the static phase of each stretch (see schema below). *B*, averaged maximal values (±S.E.M.) of burst frequency (*a*), spike frequency within bursts (*b*) and their duration (*c*) as a function of different amplitudes of muscle stretch. Each point is a mean of at least 10 consecutive responses. Asterisks indicate first test values that are significantly different ($P < 0·01$; Mann–Whitney rank sum test) from reference (stretches of 0·3 mm). *A* and *B* are from the same preparation.

DISCUSSION

We have demonstrated in the present study that a lobster primary sensory neurone, in addition to an autogenic repetitive spiking capability, is able to express rhythmic burst firing due to intrinsically driven oscillations in membrane potential. The oscillatory mechanism, which is both voltage- and mechanosensitive, is located in each of the bilateral dendritic arbors of the receptor, which in turn drive bursting in the single medial axon of the cell. Our results also show that, while AGR bursting may occur spontaneously, its expression in tonically active receptors can be enabled by modulatory influences, one of which is a member of the FMRFamide family, neuropeptide F1. These main findings are summarized in Fig. 10.

Primary sensory oscillators

The ability of neurones to oscillate endogenously is now known for a variety of cell types throughout the central nervous system (Llinás, 1988). In addition to being responsible for generating rhythmic motor behaviours (for recent review, see Marder & Calabrese, 1996), oscillatory processes appear to play significant roles in other brain functions such as sleep and attention (Steriade, McCormick & Sejnowski, 1993), as well as the central processing of visual (Gray & Singer, 1989) and olfactory (Laurent & Naraghi, 1994) sensory information.

Oscillations and burst discharge also occur in peripheral sensory pathways, having been first reported with extracellular recordings from thermoreceptors in mammalian skin (Iggo, 1969) and more recently, for second-order afferents in the electrosensory system of fish (Braun *et al.* 1994). There are precedents for intrinsic resonance phenomena in primary mechanoreceptors. For example, oscillations have been observed in the inner hair cells of goldfish (Sugihara & Furukawa, 1989) and chick (Fuchs *et al.* 1988), while a so-called 'intrinsic rhythm generator' has been proposed to underlie non-linear plateau patterns of discharge in the sensory terminals of frog muscle spindles (Sokabe *et al.* 1993). A fundamental difference between these primary mechanoreceptor rhythms and that of the AGR, however, is that they appear to involve a high-frequency (50–200 Hz) resonance mechanism which is strictly stimulus dependent and therefore inextricably linked to encoding processes in the sensory terminals. In contrast, the oscillating membrane potential of the AGR

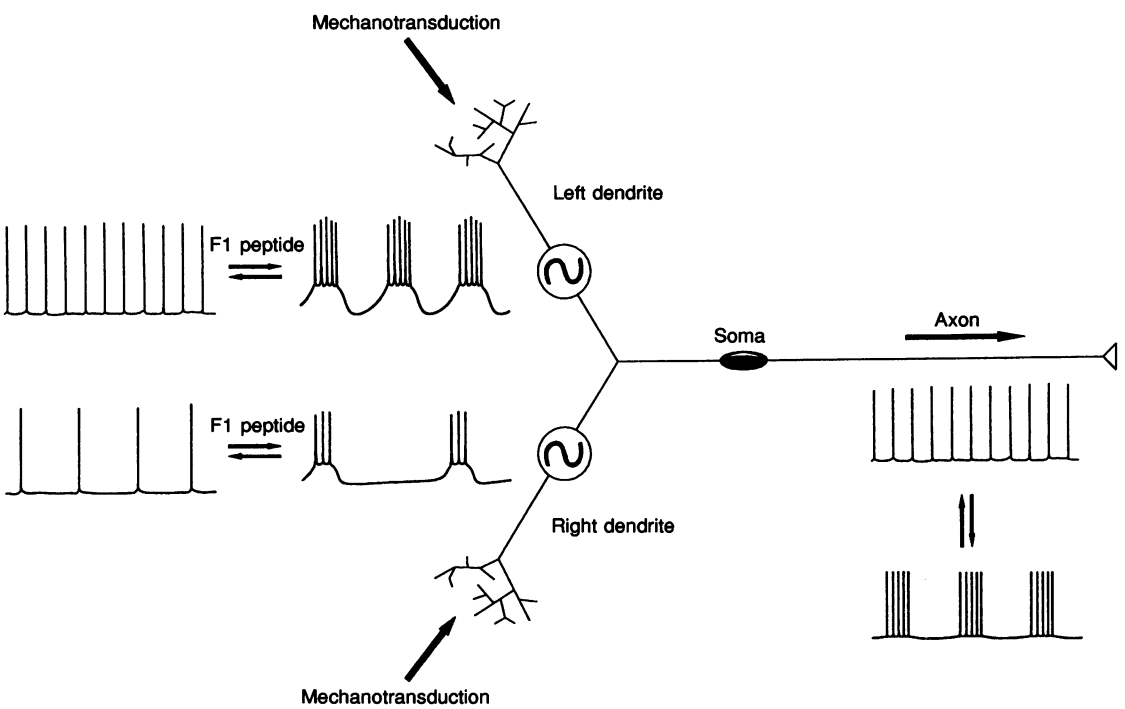


Figure 10. Summary diagram of AGR electrophysiological properties associated with autogenic tonic firing and conditional dendritic bursting

The left and right dendrites of the AGR each possess an endogenous oscillatory mechanism that can spontaneously and independently switch back and forth between two autogenic firing patterns: tonic discharge or rhythmic bursting. Both modes of activity converge onto the receptor's soma and single axon, the dendrite with the higher intrinsic excitability tending to impose its own pattern on that of its contralateral partner. The two dendritic oscillators are also chemodependent in that the transition from tonic to burst firing can be elicited separately in the two dendrites by exogenous application of an FMRFamide-related peptide, F1. Under these conditions, encoding of mechanotransduction in the left and right terminal dendrites of a bursting AGR is complex, involving alterations in the frequency, intensity and duration of on-going bursts.

occurs at much slower cycle frequencies (1–2 Hz), underlies discrete and stereotyped bursts of impulses and occurs spontaneously and separately from the sites of mechano-transduction.

Dendritic origin of autogenic bursting

In principle, autogenic bursting in the AGR could arise from the collective operation of an assemblage of voltage-sensitive channels distributed along its dendritic, soma or axon hillock membranes. Alternatively, oscillatory discharge could result from some form of electrotonic interaction between these spatially separate regions. In this context, several examples have been reported where the firing of spike doublets or impulse bursts can arise from retrograde invasion and reverberation between different compartments of the same cell (Calvin & Hartline, 1977; Turner *et al.* 1994). However, a number of observations suggest that a similar mechanism is not responsible for the mechano-receptor bursting described in the present study but rather, and in common with a number of autoactive central neurones (Llinás, 1988), this oscillatory property resides in each of the peripheral dendrites themselves. Firstly, in multiple site recordings from a bursting AGR, each spike potential recorded in the soma (and in turn the axon) is always preceded at constant latency by an impulse occurring in one or other of the receptor's two bilateral dendrites. This is not compatible with some form of reverberating interaction at the dendro-dendritic or dendro-somatic interfaces. Secondly, the dendritic bursts remain unaffected by blockage of axonal action potentials with intrasomatic hyperpolarizing current injection or focal application of TTX, although their rhythm can be phase reset by brief electrical stimulation of the corresponding dendrite. Thirdly and most compellingly, both dendritic processes continue to burst spontaneously at their inherent frequencies when physically disconnected from each other and from the soma and axon of the receptor.

Since the AGR encodes information from two spatially separate dendritic fields (each associated with different bundles of the GM1 muscle), an oscillator at each of these distant sites offers a synchronizing mechanism by which bilateral input from the receptor terminals can be co-ordinated into a single coherent pattern before its transmission to the initial axon segment of the cell. Whichever terminal has the higher intrinsic excitability or is subjected to the greater mechanical stimulation will dominate, its individual impulses or spike bursts invading the contralateral terminal and resetting that terminal's own pacemaker cycle. Interestingly, a very similar mechanism involving competitive pacemaker interactions between different primary afferent endings has been reported in mammalian muscle spindles (for recent review, see Banks, Hulliger, Scheepstra & Otten, 1995).

It is also important to note that the expression of bursting behaviour by the AGR is not in some way due to cell damage in our *in vitro* experimental conditions, since it also displayed this property in suction electrode recordings from semi-intact preparations in which the receptor and its muscles remained intact. Moreover, an earlier study with extracellular recordings from intact crab (Heinzel, 1990) has shown that the AGR can indeed fire rhythmic bursts of action potentials *in vivo*, while endogenous burst firing in other muscle proprioceptor cell types has been reported in the crab stomatogastric system (Katz, Eigg & Harris-Warrick, 1989).

Chemodependence of mechanoreceptor rhythmicity

It is now well established that a variety of invertebrate mechanoreceptors are modulated directly by neuroactive substances at the peripheral level, before afferent information reaches the central nervous system. For example, sensory neurones from the oval organ, a stretch receptor in crustacean ventilatory appendages, are sensitive to serotonin, octopamine and the pentapeptide proctolin (Pasztor & Bush, 1989). Proctolin facilitates receptor depolarization and hence spike generation, whereas octopamine and serotonin depress receptor firing. In walking limbs of crayfish, both serotonin and proctolin enhance stretch receptor responsiveness (El Manira, Rossi-Durand & Clarac, 1991), as does octopamine when applied to stretch receptors in the wings of locusts (Ramirez & Orchard, 1990).

Unlike these cases, modulation of the mechanoreceptor AGR by the octapeptide TNRNFLRFamide involves more than a seemingly straightforward change in sensitivity to mechanostimulation, but leads to a fundamental alteration in the cell's own intrinsic bioelectrical behaviour: a switch from autoactive tonic firing to rhythmic bursting. Since this change can be induced by exogenous F1 in AGR dendrites isolated from both their central cell processes and the muscle in which they terminate, the transition between activity states must be due to a modulation of ionic conductances in the distal membrane of the sensory cells themselves.

Although we do not know the target mechanism responsible for the F1-induced switch in AGR firing patterns, this type of bimodal flexibility is now well established for a variety of neurones in the central nervous system, ranging from mammalian thalamocortical cells (McCormick & Pape, 1990*a*), for example, to elements of vertebrate and invertebrate motor pattern-generating networks (Grillner *et al.* 1991; Kiehn, 1991; Harris-Warrick *et al.* 1992). In these cases also, the transition between alternative activity states is controlled by the extrinsic neuromodulatory environment which, depending on changing computational demands, can enable (or disable) expression of membrane oscillation and bursting via activation of second messenger pathways. Interestingly, frog muscle spindle afferents have recently been found capable of bimodal patterns of excitability, with the switch from a graded to a non-linear plateau mode and vice versa probably being conditional upon calcium-regulated protein phosphorylation (Sokabe *et al.* 1993).

TNRNFLRFamide, first purified from *H. americanus* (Trimmer *et al.* 1987) is already known to promote rhythmo-

genesis at two other levels in the crustacean stomatogastric system. Bath-applied F1 elicits myogenic oscillations and contractions in stomatogastric muscles of shrimp (Meyrand & Marder, 1991) and, more recently, the peptide was shown to activate crab pyloric and gastric motor rhythms, again via the induction of oscillatory properties (Weimann, Marder, Evans & Calabrese, 1993). In the latter study, the threshold concentration of exogenous F1 which turned on motor rhythms was around 10^{-10} M, some two orders of magnitude lower than we found necessary to switch on bursting in the AGR. Since we do not know if the F1 peptide from *H. americanus* has an identical homologue in its European relative, *H. gammarus*, used in our experiments, it may be that these apparent potency differences reflect variability in the endogenous peptide of the two species. Alternatively, these threshold differences may relate to a real disparity in physiological sensitivity and action. For example, our experiments have not addressed the possibility that the onset of AGR bursting constitutes the ultimate overt expression of a sequence of modulatory changes already taking place at the subcellular level at lower peptide concentrations. In this context, it is important to remember that, unlike motor rhythm-generating neurones for example, the AGR is still performing a functionally significant role when not expressing bursting activity. Therefore, a relatively high threshold, all-or-none switch between tonic and burst firing would allow the two autogenic modes of the receptor to encode mechanosensory information over separate and clearly defined ranges of peptide concentration.

In principle, an *in vivo* modulatory action of the peptide on the dendrites of the AGR could be achieved by two pathways: via release into the haemolymph as a neurohormone, or liberation as a neurotransmitter from nearby peptide-containing neurons. Indeed there are a number of indications that both mechanisms may be involved. For example, in Crustacea, neuropeptide F1 is manufactured and released by the neurohaemal pericardial organ (Trimmer *et al.* 1987), and the position of the stomatogastric system, just anterior to the heart and pericardial plexuses (the stomatogastric ganglion actually lies within the ophthalmic artery), places the AGR in an ideal position to be influenced by circulating neurohormones. Furthermore, there is immunocytochemical evidence that at least one neurone containing FMRFamide-like peptide projects into the vicinity of the dendrites of the AGR where they terminate in the gastric GM1 muscle (P. Meyrand & D. Combes, unpublished observations). On anatomical grounds, therefore, the receptor also appears to have a nearby source of endogenous peptide for direct peripheral neuromodulation.

Functional implications of an oscillating mechanoreceptor

At the present time, the functional significance of oscillatory properties in the dendrites of the AGR remains a matter of speculation, although presumably the expression of bimodal activity patterns and the ability to switch between the two has major consequences for the computational ability of the cell. In effect, the autogenic dendritic response properties provide the AGR with an inbuilt output selection (repetitive firing or bursting) so that the role of mechanical input becomes one of shaping these pre-programmed output patterns rather than actually creating them. Whereas in a tonically active AGR this extrinsic influence is achieved via a straightforward linear frequency modulation of on-going impulses, when the receptor is bursting, incoming mechanical information is now encoded simultaneously in three different parameters of pacemaker activity (burst frequency, duration and impulse intensity), thereby enhancing considerably the information content and the precision with which the receptor can signal. In contrast to the changes seen in the duration of AGR bursts and their spike frequency during stretch of the distal terminals of the receptor, these pacemaker parameters remain relatively unaffected by continuous depolarizing current injection into the soma. This suggests therefore that, unlike most classical mechanoreceptors, the transduction process involves influences on the intrinsic oscillator mechanism of the AGR in addition to merely changing mean levels of membrane potential.

Whether the ability to encode multiple messages within a single temporal train renders any functional advantage to the AGR itself remains to be seen. Remarkably similar intrinsic oscillatory activity reported in shark ampullary neurones allows these receptors to signal modality-specific information in response to different thermal and electrical stimuli (Braun *et al.* 1994). In a somewhat analogous manner, therefore, it could be that a multiple encoding capability permits the AGR to faithfully signal one sensory modality, such as global tension changes in the foregut wall, while retaining exquisite sensitivity to another, such as phasic stimuli due to rhythmic GM1 muscle contraction. It is noteworthy in this context that the AGR does indeed appear to play a position-detecting role in the intact animal as well as monitoring active muscle forces during motor-driven contractions (Combes *et al.* 1995*b*).

In addition to controlling incoming information gathering and processing, the intrinsic oscillatory properties of the AGR could have major consequences for its postsynaptic targets. For example, neurotransmitter release can be potentiated more by short rapid presynaptic bursts than by regularly spaced impulses (Gillary & Kennedy, 1968). A similar activity-dependent variability, in combination with the capacity of the AGR to switch between tonic and burst firing patterns, does indeed appear to play a pivotal role in the contribution of the receptor to sensorimotor integration (Combes *et al.* 1995*a*). Recent experiments (to be reported fully in a subsequent paper) have revealed that, due to different facilitating capabilities of the synapses between the AGR and two follower interneurones, an excitatory disynaptic pathway to the gastric motor pattern-generating circuit is favoured during tonic firing of the receptor, whereas an alternative inhibitory pathway becomes dominant

when the AGR switches to its bursting mode. Effectively, therefore, the expression of different activity states provides this primary mechanosensor with a versatile framework by which it can route afferent information to a distant motor network target.

In conclusion, the present results add an important new dimension to previous experiments (Simmers & Moulins, 1988*a*; Combes *et al.* 1993), providing evidence that the dendrites of this single primary mechanoreceptor neurone possess membrane properties far removed from those expected of simple linear cables. These intrinsic dendritic mechanisms, which are not 'hard-wired' but appear to be susceptible to a switching on and off process by the immediate modulatory environment, introduce considerable non-linearity in the transduction and encoding processes, thereby imparting an integrative capability expected only of more complex multicellular systems.

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Acknowledgements

We thank Dr Richard Miles for helpful discussions and comments on the manuscript. This work was supported by the Human Frontier Science Program and a doctoral studentship from the Ministère de la Recherche et de l'Espace to D.C.

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Received 25 July 1996; accepted 5 November 1996.