J. exp. Biol. **114**, 521–549 (1985)

MOTOR PROGRAMME SWITCHING IN THE CRAYFISH SWIMMERET SYSTEM

BY W. J. HEITLER

Department of Zoology, University of California, Davis, California, U.S.A. and *Department of Zoology, University of Oxford, England* and *Gatty Marine Laboratory, University of Saint Andrews, Fife, KY16 8LB, Scotland**

Accepted 14 August 1984

SUMMARY

1. Intracellular and extracellular recordings have been made from neurones of the swimmeret system in the semi-isolated abdominal ganglion of the crayfish during rhythmic activity.

2. Extracellular recordings commonly reveal a motor programme (MP1) consisting of low-amplitude symmetrical power and return stroke activity with phase-constant posterior-to-anterior intersegmental coordination. Occasionally a different motor programme (MP2) is expressed. MP2 has higher amplitude episodic activity, with return stroke duration greater than power stroke, and with latency-constant anterior-to-posterior or near synchronous intersegmental coordination. Preparations may switch spontaneously between the two motor programmes.

3. Intracellular recordings show that interneurones whose membrane potentials oscillate during MP1 and which can reset its rhythm usually cease to oscillate during MP2.

4. During production of MP1, current injected into any one of a small number of interneurones can induce MP2. The polarity of current required is usually such as to drive the membrane potential towards the level normally associated with return stroke during MP1.

5. During MP1 many motor neurones receive synaptic input with approximately sinusoidal waveform. During MP2 they may receive an episodic input with approximately sawtooth waveform, and/or input consisting of large, unitary EPSPs. The unitary EPSPs drive a 'bursty' mode of MP2 activity that is sometimes seen.

6. The bursts of unitary EPSPs in MP2 appear to derive from a different source to that of the sinusoidal input in MP1. These sources are probably caudally-conducting through-interneurones and non-spiking local interneurones respectively.

7. Thus experimental perturbation of a single neurone can induce a motor programme switch such as to change the activity of some hundreds of neurones in at least three ganglia. Neurones with this property would be convenient targets for controlling influences in the intact animal.

* Present address and address for reprints.
Key words: Crayfish, swimmeret, motor programme, switching, coordination, CPG.

INTRODUCTION

Most, if not all, of the rhythmic behavioural sequences of animals result from the interaction of central pattern generators (CPGs; for reviews see Roberts & Roberts, 1983) with sensory feedback. The term 'CPG' refers to an ensemble of central neurones (minimally a single neurone, but more probably a network of neurones) whose individual properties and interactions produce coordinated sequences of rhythmic activity in motor neurones. A stereotyped pattern of such activity is called a 'motor programme'. Some types of behaviour can be driven by more than one motor programme, as in the different gaits of many locomotory activities. Each gait has distinct amplitude, frequency and coordination characteristics. Thus in the cat the forelimbs act in phase during galloping or jumping, but in antiphase during walking, trotting or swimming (Miller, van der Burg & Meche, 1975*a*,*b*). Similarly in crabs and lobsters, changes in the direction of walking are accompanied by shifts in inter-limb coordination and changes in the relative intensity of activity in different muscles (for reviews see Evoy & Ayers, 1982; Wiens, 1982). Many further examples could be drawn from non-locomotory behaviour such as feeding (McClellan, 1978; Croll & Davis, 1981, 1982), ventilation (Arudpragasam & Naylor, 1966; Simmers & Bush, 1983*b*), heartbeat (Calabrese & Peterson, 1983), sound production (Bentley, 1977), etc. Several questions of general interest arise from a consideration of such changes in motor programme. These include the nature of the mechanism which allows the CNS to produce more than one motor programme (in particular whether each programme is driven by a separate CPG), whether the same motor neurones are involved in different programmes, and how switching is brought about between programmes.

One of the first systems in which a CPG was demonstrated was that of the swimmerets of crayfish (Hughes & Wiersma, 1960; Ikeda & Wiersma, 1964). Swimmerets are the segmentally-paired, biramous paddles on the ventral surface of the abdomen, which beat back and forth during swimming, walking, burrow ventilation and ventilation of eggs on a gravid female (Huxley, 1880). Each swimmeret is controlled by a neural mechanism located within the hemiganglion of its segment and coupled to the other segments. A lobster swimmeret motor programme has been described in detail using data derived from extracellular recordings of the various motor neurones (Davis, 1968*a*,*b*, 1969, 1971). The CPG producing the programme could be modelled as a sinusoidal oscillator driving about 50 motor neurones to 11 muscles. This produces alternating power and return stroke movements on which are superimposed other control movements. Segments are bilaterally synchronized and coordinated in a rear-to-front metachronal wave, which is approximately phase-constant over a range of cycle periods (Davis & Kennedy, 1972). Recent studies on intact lobsters have shown that this sinusoidal activity is characteristic of the swimmerets when they beat in the non-walking lobster, but that a more episodic output with a different coordination mode is obtained when the swimmerets beat during walking (Cattaert & Clarac, 1983). Thus intact lobsters can switch between at least two programmes for swimmeret beating.

In this report swimmeret motor programme switching in the isolated abdominal

nerve cord of the crayfish *Pacifastacus leniusculus* is described. The most common motor programme observed is similar to that described originally for the lobster (Davis, 1968*a*,*b*, 1969). However, at least one and possibly several other output patterns have been found which differ in frequency, amplitude and coordination mode from the common pattern. The motor output may switch spontaneously between different patterns. Intracellular recordings from the motor neurones and interneurones of the system show that switching is usually accompanied by a large change in the activity of interneurones intimately involved in production of the motor patterns. Some of these interneurones are able to switch the pattern when injected with small amounts of current. It is concluded that there are subsets of interneurones which are differentially active in the various forms of motor output. At least some motor neurones receive input from all known subsets; the particular motor programme expressed depends on which subset is active.

MATERIALS AND METHODS

Experiments were performed on the abdominal central nervous system of the crayfish *Pacifastacus leniusculus*. Animals were obtained from Riversdale Farm (Stour Provost, Near Gillingham, Dorset, England), where they are reared in a natural environment. The ventral nerve cord (VNC), including the chain of ganglia from the 3rd to the 6th (hereafter referred to as g3, g4, g5 and g6), were removed from the abdomen and pinned dorsal surface upwards on a Sylgard platform placed in a Petri dish filled with Van Harreveld's crayfish saline. The telson and uropods were left attached to g6 and transferred to the dish with the VNC, but otherwise the CNS was isolated from the periphery. The VNC was slightly stretched as it was pinned out. These procedures seemed to increase the probability of spontaneous rhythmic swimmeret motor activity, but this was not studied systematically. No attempts were made to induce rhythmic activity by stimulation or pharmacological agents. Pin electrodes were used to record from the anterior and/or posterior branches of the 1st roots of g3, g4 and g5. The 1st roots innervate the swimmerets, with the axons of power stroke (retractor) motor neurones mainly running in the posterior branch, and the axons of return stroke (protractor) motor neurones mainly running in the anterior branch (Davis & Kennedy, 1972). Unless specified otherwise, recordings were made from the right side of the animal. Extracellular recordings were from the whole 1st root, unless specified as being particularly from the anterior or posterior branch. In most preparations the periganglionic sheath was removed from the dorsal surface of g4, and glass microelectrodes were used to record from and inject current into various neurones intracellularly. The electrodes had resistances in the range 40–90 MΩ, and were filled with either 3 mol l^{-1} potassium acetate, or 5% Lucifer Yellow dissolved in 1 mol l^{-1} lithium chloride (Stewart, 1978).

Motor neurones were identified by correlation of intracellularly and extracellularly recorded spikes, and by the occurrence of antidromic spikes on stimulating the 1st root. If separate extracellular recordings were made from the anterior and posterior branches of the 1st root, motor neurones could be characterized according to the branch in which their axons ran. Recordings from a few preparations in

which a swimmeret was left attached to a ganglion showed the power stroke phase (retraction) to be accompanied by higher amplitude activity in the 1st root than return stroke (protraction). Similar phases of large and small amplitude could often be distinguished in recordings from the isolated preparation. Recordings from the 1st root of an isolated swimmeret show sensory spikes resulting from swimmeret movements to be low amplitude, so the high amplitude phase of motor output from the isolated CNS probably consists of power stroke, and the low amplitude phase of return stroke. Motor neurones could thus also be characterized according to the phase in which they were active. Many motor neurones, such as ramus spreaders and twisters, are neither true power stroke nor return stroke neurones. However, most are active in one or the other phase, and thus can conveniently be categorized in those terms. Interneurones were identified as those neurones which had no correlated peripheral spike or antidromic spike. This identification was sometimes confirmed anatomically by subsequent Lucifer Yellow dye injection.

Intracellular recordings were made from neuropilar regions of neurones, because recordings from cell bodies revealed only very small, attenuated potentials. Neurones were thus penetrated 'blind', since few landmarks beyond the general shape of the ganglion are available to aid in locating particular neurones. Some neurones, such as the non-spiking stretch receptors (Heitler, 1982) or the segmental giant (Kramer, Krasne & Wine, 1981), can be aimed for with a high probability of success, and unambiguously identified once penetrated. Such neurones are, however, a minority of the swimmeret system. Even after a swimmeret neurone has been characterized physiologically and stained by intracellular dye injection it is difficult in most cases to be sure of returning to the same neurone in a different preparation. There are three problems. First, as described in this paper, different preparations can exhibit different types of activity, and physiological characterization of a neurone during one type may be no help in recognizing the same neurone during expression of another type. Second, staining of two neurones using two microelectrodes, or resulting from dye-coupling after injecting a single neurone, has shown that several neurones exist with *very* similar anatomy. Thus a neurone cannot be identified unambiguously on the basis of its anatomy. Third, many of the neurones of interest have neuropile processes no greater than 4–8 μm in diameter, and such neurones are difficult to locate consistently, even if they can be recognized once located. The overall effect of these problems has been that while experiments may be replicated several times (according to the duration of the microelectrode penetration) *within* a particular preparation, it has been difficult to repeat exactly the same experiment on the same individual neurones in different preparations. Conclusions are thus based on the weight of evidence deriving from somewhat varied experiments, and the limitations inherent in this procedure are recognized.

RESULTS

The 'normal' motor programme

Approximately 300 preparations have been examined, most of which had either tonic output, or quasi-rhythmic output that was too variable and irregular to allow

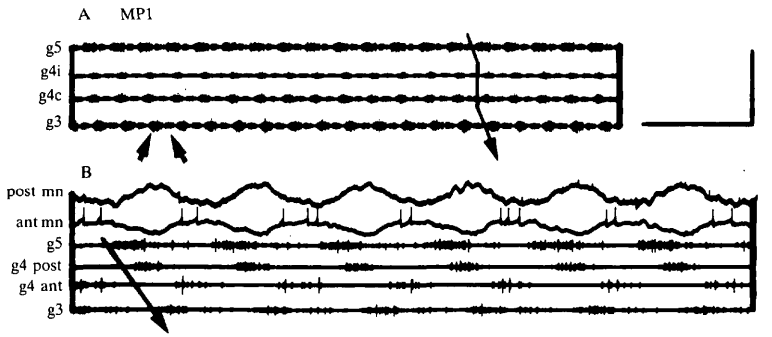

Fig. 1. (A),(B) The 'normal' motor programme: MP1. (A) Slow time base recording of extracellular motor activity displaying the swimmeret rhythm in g5 (1st trace), g4 (2nd trace), contralateral g4 (3rd trace), g3 (4th trace). Note power and return stroke bursts (1st and 2nd arrowheads respectively, 4th trace) can be distinguished by the greater amplitude of the former. The difference is not always so clear as in this example. The normal posterior-to-anterior metachronal and bilaterally synchronous coordination is displayed (oblique and vertical arrow). (B) Faster time base recording showing intracellular recordings from a g4 posterior branch motor neurone (1st trace), g4 anterior branch motor neurone (2nd trace), and extracellular recordings from g5 (3rd trace), g4 posterior (4th trace), g4 anterior (5th trace) and g3 (6th trace). The intracellular recordings display approximately antiphasic sinusoidal oscillations, subthreshold in the posterior motor neurone. The extracellular recordings show the normal interganglionic metachronal coordination (oblique arrow). Calibration: horizontal (A) 3·5 s, (B) 0·8 s; vertical (B) 1st trace 20 mV, 2nd trace 50 mV.

characterization. However, one very stable form of output, arbitrarily called motor programme 1 (MP1), has been recorded in a total of 53 preparations (Fig. 1). This rhythm appears homologous to that described for the non-walking lobster. In a healthy preparation MP1 may continue with little interruption for several hours, varying in frequency by only a few percent over that period. It consists of alternating bursts in power and return stroke motor neurones normally coordinated in approximate antiphase at frequencies in the range 0·7–1·5 Hz. Power stroke activity is usually stronger, as judged by number and frequency of units active within a burst, but the duration of power and return strokes are approximately equal within each cycle. Bilateral output from a single segment is usually synchronized, while output from adjacent segments is coordinated in a posterior-to-anterior metachronal wave, with a phase lag of about 20% between segments. Many, although not all, of the motor and interneurones active during MP1 display approximately sinusoidal membrane potential oscillations. Motor neurones which spike during MP1 mainly produce slow and rather weak contractions of the swimmeret muscles. If the swimmerets are left attached to an abdomen which is isolated from the cephalothorax, MP1 is expressed as gentle wafting movements in which the metachronal coordination is clearly visible.

Different motor programmes

MP1 is the most common stable swimmeret output expressed by the isolated

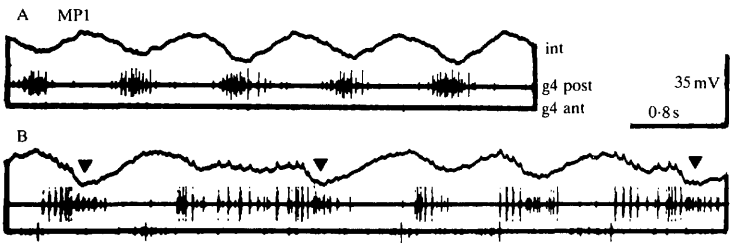

Fig. 2. (A),(B) Disruption of MP1 by irregular, quasi-rhythmic activity. Intracellular recording made in g4 from an apparently non-spiking interneurone (int, 1st trace), extracellular recordings from the posterior (post, 2nd trace) and anterior (ant, 3rd trace) branches. (A) The interneurone oscillates with approximately sinusoidal waveform during MP1, hyperpolarized during the power stroke phase. Anterior branch motor neurones do not spike. (B) MP1 is interrupted by bouts of discoordinated motor output. The interneurone still hyperpolarizes in phase with bursts of relatively small spikes in the posterior branch (triangles), but depolarizes with discrete EPSPs which are synchronous with the brief bursts of large spikes in the posterior branch.

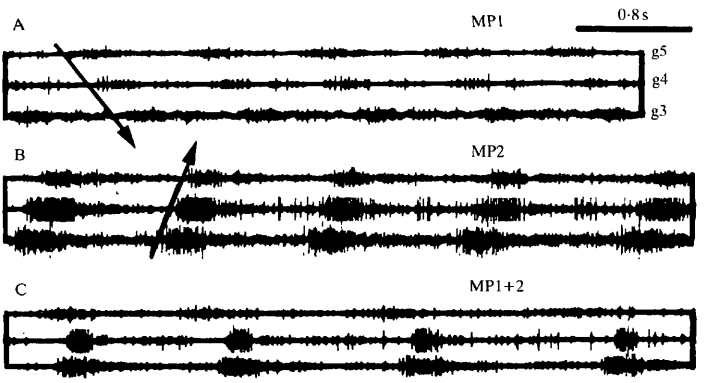

Fig. 3. (A)–(C) Comparison between the common motor programme MP1 and an uncommon programme arbitrarily called MP2. Extracellular records from g5 (1st trace), g4 (2nd trace) and g3 (3rd trace). (A) MP1 displays posterior-to-anterior metachronal coordination (oblique arrow) and a relatively low amplitude of motor output. (B) A bout of MP2 from the same preparation as (A). The metachronal coordination is reversed relative to MP1 (oblique arrow), the amplitude of motor output is considerably increased, and the frequency is reduced. Note the 'bursty' structure occasionally apparent (e.g. in the 4th power stroke cycle of g4). (C) A mixed motor programme from another preparation; g3 and g4 express MP2; g5 has the amplitude and coordination relative to g4 of MP1, but the period of MP2. Note how the period increases throughout the bout. g5 remains approximately phase-constant to g4, but g4 is latency-constant to g3.

CNS. It is highly stereotyped, and can usually be identified without ambiguity when it occurs in different preparations. Output can also occur which is clearly distinguishable from MP1 in terms of amplitude, and/or frequency and/or coordination mode. Sometimes this output is too irregular and uncoordinated to be characterized (Fig. 2). However, other forms are sufficiently stereotyped and regular to be recognized as distinct motor programmes (Fig. 3). These programmes are more variable than MP1, but can be distinguished from it by one or more of the following characteristics (given in descending order of diagnostic certainty). Instead of rear-to-front metachronal coordination, there is either front-to-rear metachronal or, less frequently, approximately synchronous coordination. More units are active at a higher intra-burst frequency, and the recruited units have larger amplitude spikes in extracellular recordings. Sometimes these units fire bursts within each cycle period. The intersegmental metachronal latency is short, and it, rather than the phase, is approximately constant across varying cycle periods. The duration of return stroke is usually greater than that of the power stroke, rather than equal to it. The overall cycle period is often longer and more variable.

MP1 is usually expressed by the entire abdominal chain of ganglia in a coordinated manner: the output of each ganglion is very similar, but shifted in phase relative to adjacent ganglia (Fig. 3A). The same is sometimes true of non-MP1 output (Fig. 3B), but in many preparations one ganglion (usually the terminal ganglion at either end) becomes partially or completely uncoupled from the other ganglia in terms of the motor programme it displays (Fig. 3C). Such uncoupling is apparent in the extracellular recordings prior to making intracellular recordings, and is thus not caused by damage to the CNS resulting from microelectrode penetration. A few preparations have been found in which residual elements of one motor programme are present even when the output is predominantly of another form.

As will be seen in the examples presented below, the characteristics of the non-MP1 forms of output are much more variable than those of MP1. Thus it is not clear whether they constitute a single motor programme, or a range of similar but distinct motor programmes. However, since most examples of non-MP1 output share several of the distinguishing characteristics described above, they are regarded in this report as a single, variable motor programme, arbitrarily called motor programme 2 (MP2). If the swimmerets are left attached, MP2 is expressed as vigorous, rapid movements clearly different from those of MP1. MP2 has been recorded in a total of 21 preparations. In 18 of these MP2 occurred as short bouts interrupting long sequences of MP1, while in the remaining 3 preparations MP2 was the only rhythmic motor programme expressed.

Unfortunately, in only a few preparations demonstrating motor programme switching has it been possible to make intracellular recordings from neurones involved either in the generation of the rhythms themselves, or in switching between them. However, sufficient data have been obtained to show that a switch from MP1 to MP2 can be induced in the entire chain of abdominal ganglia by injecting current into any one of several different neurones. A detailed description of the 'best' preparation showing spontaneous and induced motor programme

switching (i.e. one with the most repetitions, and the most clear-cut differences in the output) is given next, followed by a briefer description of other preparations illustrating different aspects of switching between partial, or less stereotyped forms of the programmes.

Spontaneous and induced motor programme switching: an example

Extracellular recordings were made from a preparation which expressed MP1 almost continuously for several hours, interrupted by 6- to 10-s bouts of MP2. These bouts occurred at 0·5- to 5-min intervals and were followed by a 5- to 6-s period of tonic motor activity. Simultaneous intracellular recordings were made from a power stroke motor neurone and an interneurone in g4 (Fig. 4). During MP1 the interneurone and the motor neurone displayed approximately sinusoidal oscillations, with the interneurone slightly preceding the motor neurone in phase. The chain of ganglia was coordinated in the usual rear-to-front metachronal wave. The input to the motor neurone was subthreshold for the duration of the experiment, but spiking could be induced by injecting small amounts of depolarizing current. Both depolarizing and hyperpolarizing current injected into the motor neurone affected the spike activity of other motor neurones recorded extracellularly, but did not affect the period or phase of the rhythm. The motor neurone thus had non-spiking central output, but was not intimately involved in the CPG. The interneurone could not be induced to spike with injection of up to 10 nA depolarizing current, but small amounts of current (1–2 nA) excited g4 power stroke and inhibited g4 return stroke motor neurones (as judged by extracellular recordings), while hyperpolarizing current had the opposite effect (Fig. 4A,B). In both cases the oscillations recorded intracellularly in the motor neurone appeared to stop (or at least were substantially reduced), suggesting that the CPG itself was affected. This was confirmed using short pulses of depolarizing or hyperpolarizing current (2 nA). These reset the period of MP1 in all three ganglia when injected into the interneurone at the appropriate phase of the cycle, i.e. antagonistic to the activity (Fig. 4C,D). Depolarizing current prolonged g4 and slightly truncated g5 power stroke, while hyperpolarizing current abolished g4 power stroke activity and the subsequent g3 cycle, and prolonged g4 return stroke and g5 activity. Thus the interneurone had non-spiking output within the CNS, had effects on the interganglionic coordination of the rhythm, and was intimately involved in the circuitry producing MP1.

The intracellular recordings were maintained for about 45 min, during which seven spontaneous and six induced (see below) bouts of MP2 occurred. During spontaneous bouts of MP2 the most obvious changes apparent in the extracellular recordings were the increase in amplitude and the reversal of direction of metachronal coordination which affected the entire chain of ganglia (Fig. 5). The period of oscillation slowed and some units in both the posterior and anterior branches fired in bursts within the individual cycles. The waveform of the motor neurone changed from approximately sinusoidal to a higher amplitude (although still subthreshold) sawtooth-like potential with a rapid rise and slower fall time. The cycle period increased gradually within each bout as a result of extending this fall

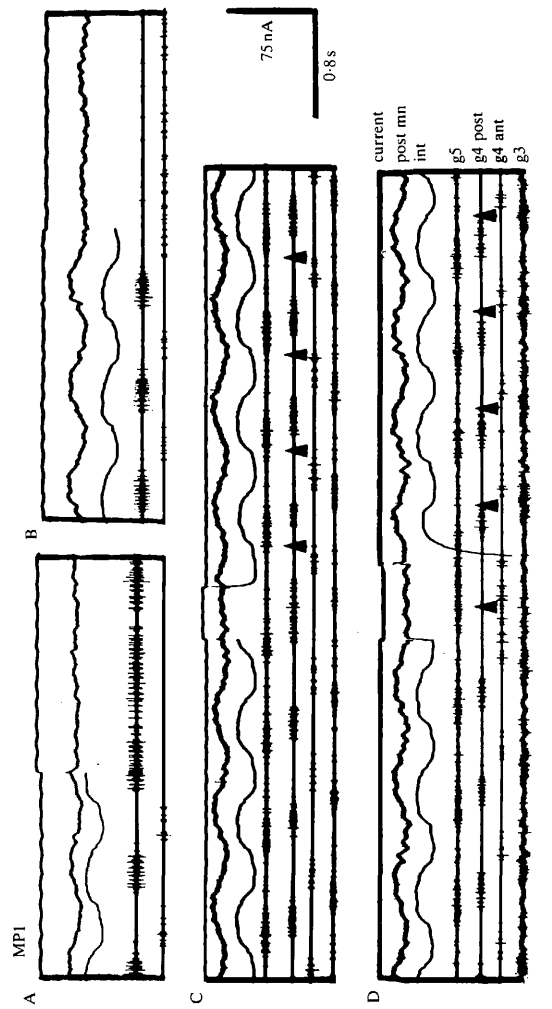

Fig. 4. (A)–(D) Characteristics of a switch-inducing interneurone (3rd trace) and a posterior branch motor neurone (2nd trace) during MP1. (A) Depolarizing current (1st trace) injected into the interneurone increases g4 posterior branch (4th trace) and decreases anterior branch (5th trace) activity. The motor neurone membrane potential stabilizes at the approximate midpoint of its oscillation. (B) Hyperpolarizing current has the opposite effect on extracellularly recorded activity, and the motor neurone stabilizes with a more hyperpolarized membrane potential. (C) A pulse of depolarizing current injected so as to extend the depolarized phase of the interneurone resets the rhythm as expressed by extracellular activity in g5 (4th trace), g4 posterior branch (5th trace), g4 anterior branch (6th trace) and g3 (7th trace). Arrowheads indicate time at which g4 posterior branch bursts would have been expected to start had no resetting occurred. (D) A hyperpolarizing current pulse which extends the hyperpolarized phase of the interneurone also resets the rhythm (arrowheads as in C). In record D the current monitor malfunctioned, and the 1st trace has been hand-drawn (to scale) to indicate the expected current from the voltage applied to the bridge circuit. The bridge was not balanced during current injection. Calibration: vertical 2nd trace 40 mV, 3rd trace 100 mV.

time, so that the sawtooth appeared episodic, rather than continuous. The motor neurone maintained the same phase relationship to the extracellularly recorded output as in MP1. The waveform of the interneurone changed considerably. The interneurone hyperpolarized, and the oscillations were markedly reduced in amplitude. Residual oscillations were inverted, so that they hyperpolarized rather than depolarized during the power stroke (although a small depolarizing potential could still sometimes be observed at the peak of g4 power stroke). It was apparent from the extracellular recordings that the switch to MP2 first occurred in the anterior g3, and then spread posteriorly, not affecting g5 until two or three cycles later. For the first 1–2 cycles after the switch in g3, the extracellular recording from g4 often displayed both the early, low amplitude burst of MP1 and the delayed, high amplitude burst of MP2. The interneurone was depolarized during the former, and then abruptly hyperpolarized during the latter. The major depolarized phase of the motor neurone was coincident with this hyperpolarization of the interneurone.

Thus in this preparation the spontaneous switch from MP1 to MP2 was accompanied by hyperpolarization of a particular power stroke excitor interneurone involved in the circuitry producing MP1. The same switch was reliably induced (six trials) by injecting the same interneurone with 3–4 nA hyperpolarizing current (Fig. 6). The bouts of MP2 induced experimentally took 1–3 s to be evoked, but once started had the same low frequency, high amplitude and reversed coordination characteristic of the spontaneous bouts of MP2. The induced MP2 was also initiated in g3, but unlike the spontaneous form no mixed output was obtained in the early cycles from g4. After the start of current injection but before initiation of MP2, activity in g4 posterior branch and g3 activity was reduced, while g4 anterior branch and g5 activity increased (e.g. Fig. 6C,D). A fortuitous (and brief) penetration of the interneurone by a second microelectrode showed that only 7–8 mV of membrane potential hyperpolarization was necessary to induce MP2. Once initiated, MP2 continued even if the interneurone was released from experimental hyperpolarization (Fig. 6D). The interneurone then showed a membrane potential hyperpolarization and waveform similar to that occurring in a spontaneous bout of MP2. The spontaneous bouts of MP2 were sufficiently widely spaced to ensure that the regular occurrence of a bout following experimental hyperpolarization of the interneurone was not coincidental, but they were too irregular to determine whether the induced bouts had any effect on the subsequent timing of the next spontaneous bout.

Fig. 5. (A)–(D) Four examples of spontaneous switching from MP1 to MP2 (same preparation as Fig. 4, traces as Fig. 4C except no current monitor). Note the switch from the 5–4–3 metachronal coordination of MP1 (D: 1st oblique arrow) to the 3–4–5 coordination of MP2 (D: 2nd oblique arrow), and the increased amplitude of output in the latter programme. During MP2 the interneurone oscillations are reduced; remaining oscillations are mainly in antiphase rather than in phase with the motor neurone (B: vertical line between intracellular traces), although small in-phase oscillations may sometimes be superimposed (preceding cycle). In the early cycles of the switch mixed output may be obtained: MP1 occurs as a small burst of spikes in g4 posterior branch accompanied by brief interneurone depolarization, and is immediately followed by MP2 expressed as a larger burst and interneurone hyperpolarization (B,C: filled triangles between intracellular traces). Note the 'bursty' structure of some cycles (e.g. C: last g4 posterior branch burst). Calibration: vertical 1st trace 40 mV, 2nd trace 100 mV.

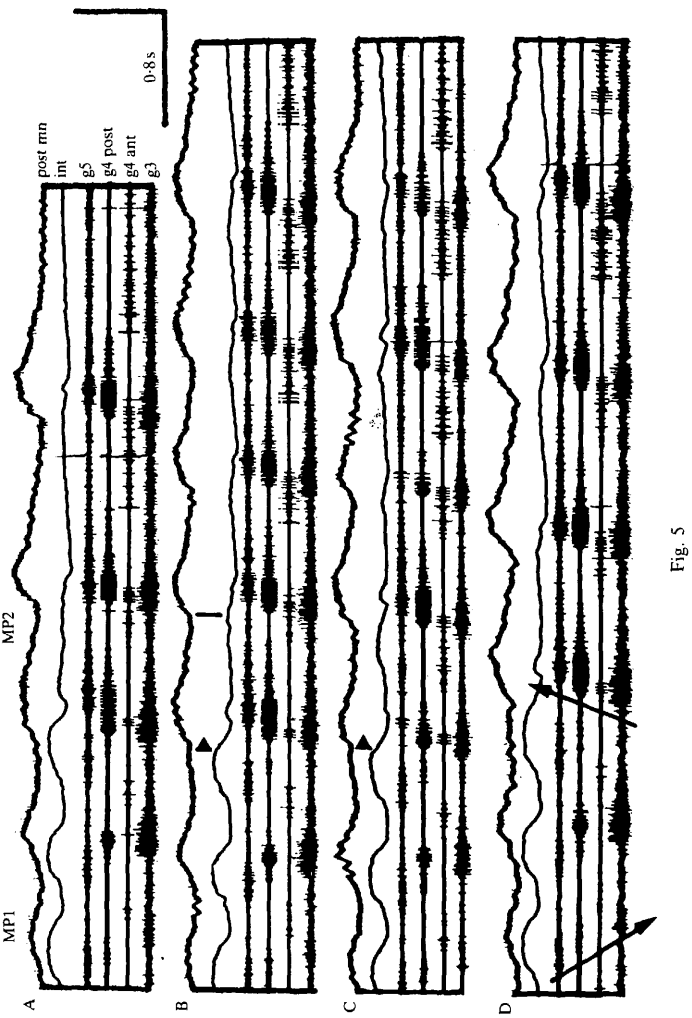

Fig. 5

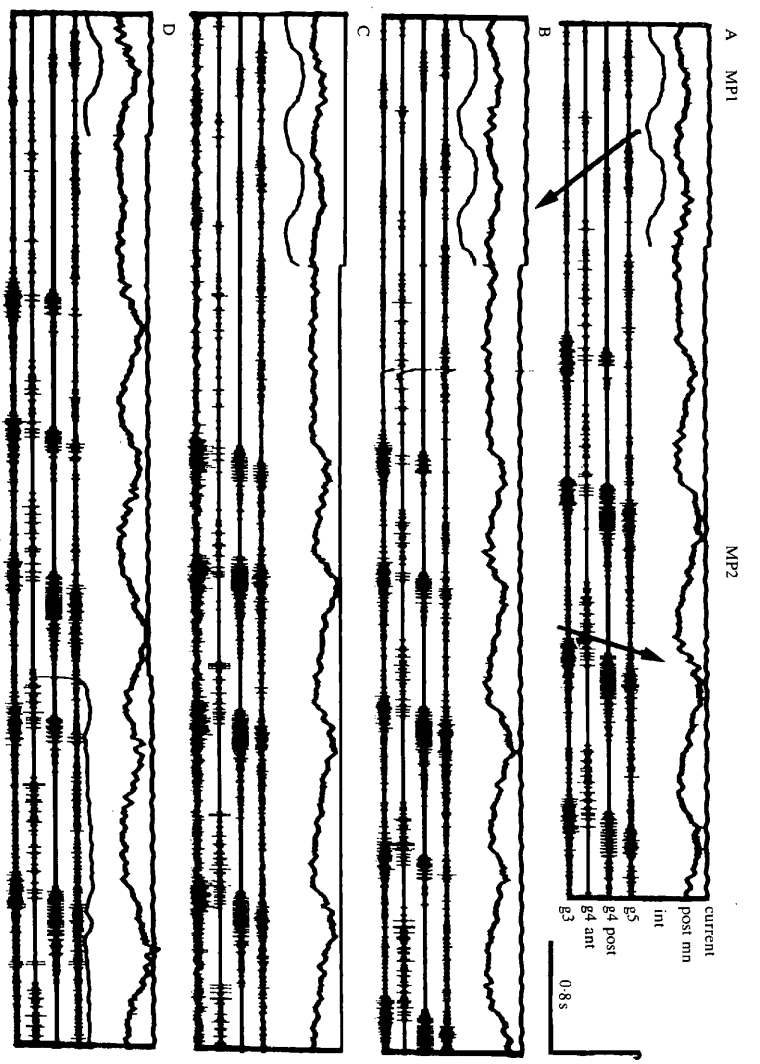

Fig. 6

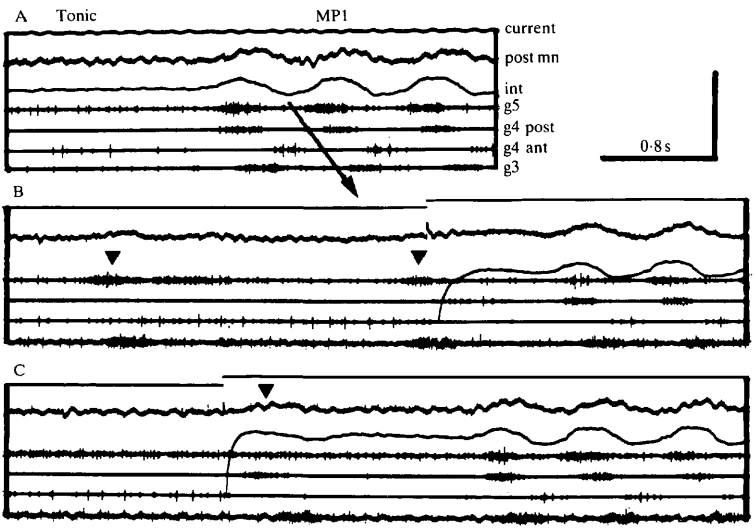

Fig. 7. (A)–(C) Resumption of MP1 after the tonic period following a bout of MP2 (same preparation as Fig. 4, traces as Fig. 4C). (A) A normal restart occurring 6 s after termination of a spontaneous bout of MP2. Full metachronal coordination (oblique arrow) is established in the 2nd cycle of MP1. (B) The interneurone is maintained hyperpolarized for about 10 s after the termination of an induced bout of MP2. Bursts in g5 and g3 apparently indicate that MP1 attempts to restart (triangles), but is unable to establish fully until the interneurone is released from hyperpolarization. (C) The interneurone is released from hyperpolarization about 2 s after termination of an induced bout of MP2, and a brief burst of activity appears in g4 posterior branch and g3 (triangle). MP1 restarts fully after a quiescent period of normal duration. Calibration: vertical 2nd trace 40 mV, 3rd trace 100 mV.

During the tonic output following MP2 the membrane potential oscillations of the interneurone and motor neurone ceased, with the motor neurone stabilized about the most hyperpolarized level of MP1 and the interneurone stabilized at the mean potential of MP1 (i.e. the mid-point of the oscillation) (Fig. 7A). In the unperturbed preparation MP1 restarted spontaneously with a simultaneous burst in g4 posterior branch and g5 after about 6 s of tonic activity. The appropriate metachronal 5–4 delay was only established in the second cycle of MP1, although the 4–3 delay was present in the first cycle. Prolonged hyperpolarization of the interneurone did not increase the duration of MP2, but prevented MP1 from restarting, so that an extended tonic period resulted. At approximately the time

Fig. 6. (A)–(D) Four examples of induced switching from MP1 (A: 1st oblique arrow) to MP2 (A: 2nd oblique arrow) in the same preparation as Fig. 4 (traces are as in Fig. 4C). (A)–(C) The duration of injected current outlasts that of the records displayed. (D) Briefer current injection shows that once MP2 has been induced it can outlast the initiating stimulus, and that the interneurone membrane potential waveform in an induced bout of MP2 is similar to that in a spontaneous bout. Calibration: vertical 2nd trace 40 mV, 3rd trace 100 mV.

when MP1 would normally have restarted; a brief burst was sometimes observed in g5 and g3 and a small depolarization occurred in the g4 motor neurone, but normal oscillatory activity did not resume while the interneurone was maintained hyperpolarized (Fig. 7B). Conversely, if the interneurone was released from hyperpolarization during the tonic period before MP1 would normally resume, the tonic period continued for its normal duration, although a brief 'rebound' power stroke excitation occurred in g4 and g3 (Fig. 7C).

Other interneurones and motor programme switching

In the preparation described above, MP2 was induced by hyperpolarizing an interneurone which depolarized in phase with power stroke during MP1, and which inhibited power stroke when hyperpolarized with current below the level required to initiate MP2. Intracellular recordings have also been made from interneurones which depolarize in phase with return stroke. An apparently non-spiking interneurone of this type was recorded intracellularly in g4, with properties an approximate 'mirror image' of the interneurone described above (Fig. 8). During MP1 it displayed sinusoidal oscillations with few discrete PSPs. Pulses of 1–2 nA depolarizing current injected during its hyperpolarized phase inhibited power stroke in g4 and g3, delayed the onset of the next cycle, and reset the phase of the rhythm (Fig. 8A). Thus the interneurone was a g4 power stroke inhibitor intimately involved in the production of MP1 (it may well have been a return stroke excitor too, but recording was not made from the anterior branch of g4 in this experiment).

Occasional spontaneous bouts of MP2 were produced by this preparation (Fig. 8B). During these bouts the interneurone depolarized, with unitary EPSPs visible, in phase with the high amplitude power stroke activity in the initial part of each cycle. Following the high amplitude part of most cycles of MP2 was a lower amplitude power stroke burst similar to that of MP1, and a hyperpolarization of the interneurone, also similar to that occurring during MP1. Thus elements of MP1 were apparent even when the major output was clearly MP2. Injection of 3 nA depolarizing current into the interneurone for 3–4 s during the normal production of MP1 was followed by a switch to MP2 in two out of three trials (Fig. 8C). The unsuccessful trial disrupted MP1 but did not induce MP2. Thus in this preparation, as in that described above, the switch to MP2 was accompanied by, or

Fig. 8. (A)–(C) Motor programme switching and a power stroke inhibitor interneurone. (A) A pulse of depolarizing current (1st trace) injected into the interneurone (2nd trace) during its hyperpolarized phase has little immediate effect on g5 (3rd trace) but inhibits g4 posterior branch (4th trace, g4 anterior branch not shown) and g3 (5th trace). The phase of the rhythm is reset in all three ganglia; arrowheads indicate the time at which g4 power stroke would have occurred had there been no resetting. (B) A spontaneous switch from MP1 to MP2 (i) leads to a bout of MP2, and then MP1 resumes (ii). During MP2 a high amplitude burst in g4 is accompanied by interneurone depolarization (filled triangle), and is often followed by a lower amplitude burst similar to MP1 accompanied by interneurone hyperpolarization (open triangle). Sometimes this latter phase is absent (cycle following open triangle). (i) and (ii) are continuous records. (C) A switch to MP2 follows the injection of depolarizing current into the interneurone. The induced MP2 is similar, but not identical to the spontaneous form. Note the 'bursty' structure of some of the cycles in MP2, especially the occasional approximately synchronous bursting in different ganglia (triangle).

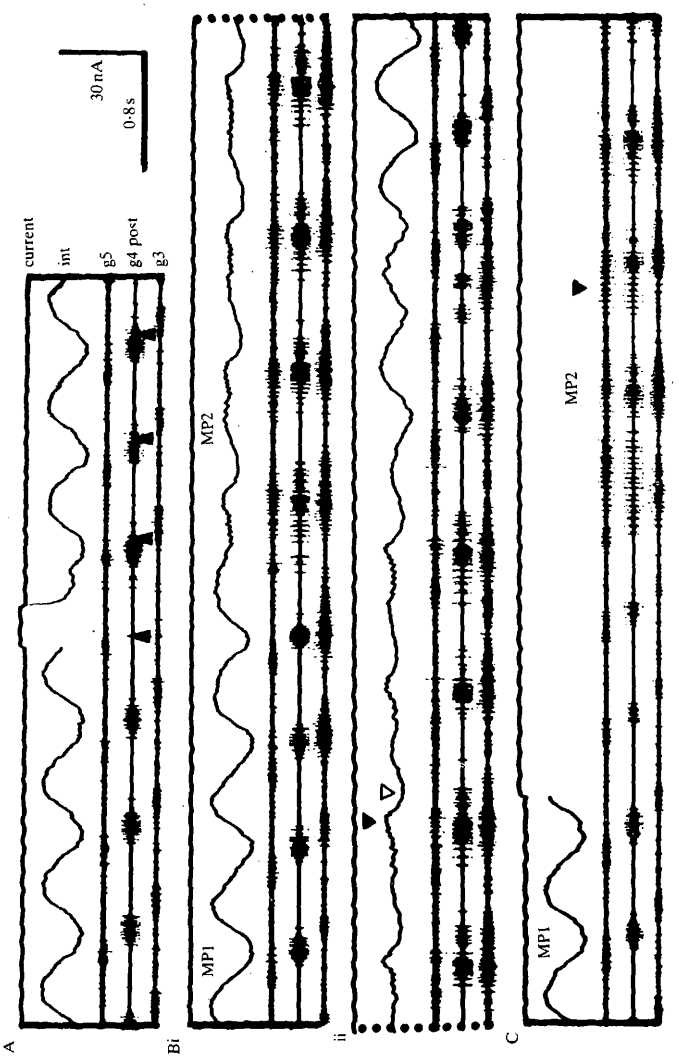

Fig. 8

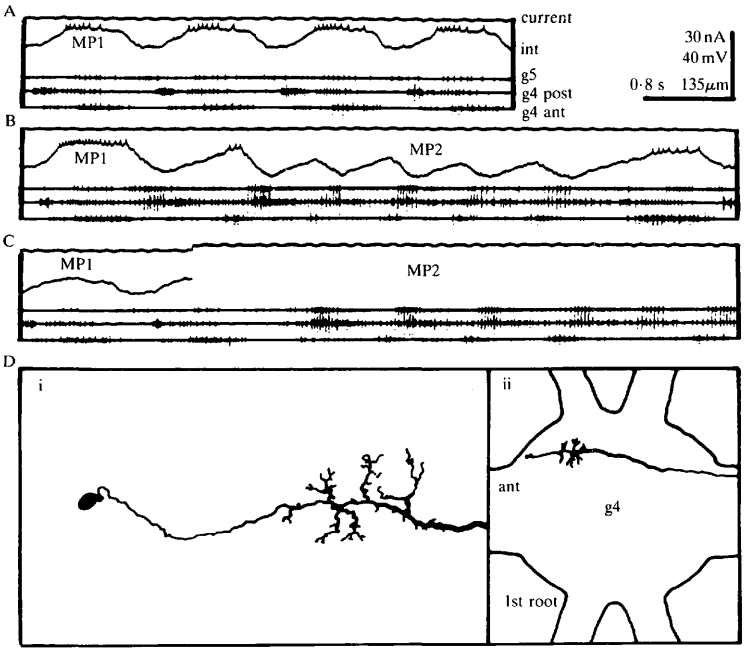

Fig. 9. (A)–(D) Spontaneous switching and switching induced by a structurally-identified g4 spiking interneurone (2nd trace). Extracellular recordings are made from g5 (3rd trace), g4 posterior branch (4th trace) and g4 anterior branch (5th trace). g3 (not shown) was not closely coordinated with g4 and g5. (A) During MP1 the interneurone spikes in phase with return stroke. (B) During MP2 the interneurone is hyperpolarized below threshold, but maintains the same phase as in MP1. (C) A switch from MP1 to MP2 induced by injecting depolarizing current (monitor in 1st trace). (D) A *camera lucida* drawing of the interneurone stained with Lucifer Yellow (i), and a sketch of the interneurone showing its location in the ganglion (ii) (ant, anterior).

induced by, perturbations of an interneurone involved in the production of MP1 such as to reduce power stroke activity. However, the variability and long latency of the MP2 bouts induced by this interneurone suggest that it is not such a powerful switch as that described above.

Neither of the interneurones described above displayed spike activity, but unfortunately their anatomy is not known. However, a spiking interneurone involved in motor programme switching has been encountered (Fig. 9). An intracellular recording with a dye-filled electrode was made from an interneurone in g4. Subsequent staining revealed a simple anatomy with a cell body in the anterior lateral ventral margin of g4, and an axon coursing directly posteriorly towards g5, close to the ipsilateral edge of the interganglionic connective (Fig. 9D). A few thin

dendritic branches arborized from the interneurone in the region where the 1st root entered the ganglion in g4.

This preparation showed spontaneous switching between a rather aberrant form of MP1 and MP2 (Fig. 9). Neither programme was fully expressed by g3. During MP1 the interneurone depolarized and spiked in phase with return stroke, which, in g4 and g5, was more prolonged than power stroke (Fig. 9A). MP2 had the characteristic high amplitude 'bursty' output which was almost synchronous in g4 and g5, but the cycle period was reduced rather than increased as is usually the case for MP2 (Fig. 9B). During MP2 the interneurone oscillated with the same phase relationship to motor output as in MP1, but with a membrane potential hyperpolarized to a subthreshold level.

Although the characteristics of MP1 and 2 in this preparation were different to those in others, they were very consistent for the duration of the experiment. Five spontaneous bouts of MP2 were observed, each with the same hyperpolarization. Injecting hyperpolarizing current into the interneurone marginally slowed the period of MP1, but did not induce a switch to MP2. However, injecting 3 nA depolarizing current caused a switch to MP2 in five out of six trials (Fig. 9C). On the one occasion when a switch to MP2 was not induced, MP1 was clearly disrupted. Thus, as in the preparations described above, the switch to MP2 was induced by producing an interneurone membrane potential change (depolarization) in the opposite direction to that normally accompanying MP1 power stroke (hyperpolarization). However, the change in this preparation was also in the opposite direction to that which accompanied a spontaneous switch, which is in contrast to the preparations described previously.

Motor neurones and motor programme switching

Are different populations of motor neurones recruited during the two programmes? The only motor neurone presented so far (Figs 4–7) did not spike during either motor programme, but did receive distinctly different forms of excitatory input, as though it might have participated in both programmes if the excitability of the preparation had been higher. Intracellular recordings were made from another g4 posterior branch motor neurone which *did* spike throughout spontaneous bouts of MP1 and 2 (Fig. 10), confirming that at least some motor neurones contribute output to both programmes. The input to the motor neurone looked similar to that described above: sinusoidal during MP1 and an episodic sawtooth during MP2. During MP2 the ramp depolarization occurred after an initial burst of spikes recorded extracellularly, suggesting that not all the motor neurones active in that programme were receiving identical synaptic input. A depolarizing current pulse injected into the motor neurone during its hyperpolarized phase reset the phase of MP1, indicating that it was involved in the generation of that rhythm (Fig. 10A). Unfortunately, the equivalent experiment was not attempted during MP2, but a hyperpolarizing pulse failed to prevent the normal occurrence of an MP2 cycle (Fig. 10B). Thus this motor neurone was involved in the generation of MP1 and contributed to its output; contributed to the output during MP2 but did not appear to be essential for it; and received the same forms of synaptic input as described previously.

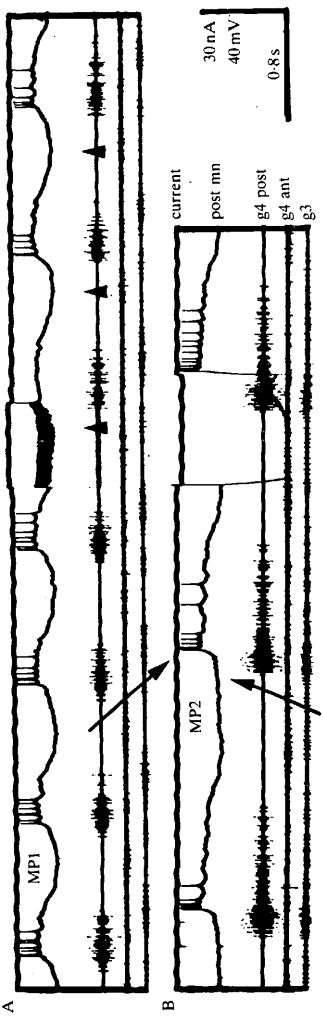

Fig. 10. (A),(B) A motor neurone which spikes in both MP1 and MP2. Intracellular recording from a g4 posterior branch motor neurone (2nd trace), with extracellular recordings from g4 posterior branch (3rd trace), g4 anterior branch (4th trace) and g3 (5th trace). (A) During MP1 the metachronal coordination is normal (oblique arrow). Depolarizing current (monitor in 1st trace) injected into the motor neurone during its hyperpolarized phase induced high-frequency spiking and reset the period of MP1 (arrowheads indicate the time at which g4 posterior branch bursts would have been expected had no resetting occurred). The extracellularly recorded motor neurone spike was very small, but is just visible as a slight thickening of the apparent baseline during the high frequency spiking (3rd trace, surrounding 1st arrowhead). (B) During MP2 the coordination reverses (oblique arrow), but the motor neurone continues to spike. Hyperpolarizing current does not disrupt the posterior branch burst.

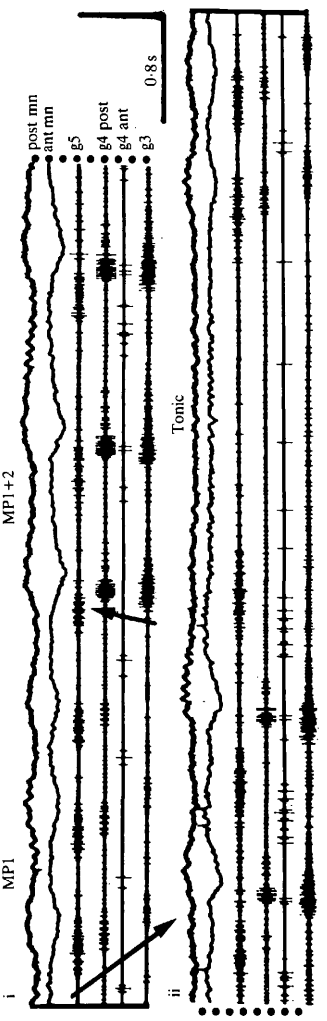

Fig. 11. Posterior and anterior branch motor neurones (1st and 2nd traces respectively) receive reciprocal inputs during both MP1 and MP2. A continuous record is shown as (i) and (ii). Extracellular recordings are from g5 (3rd trace), g4 posterior branch (4th trace), g4 anterior branch (5th trace) and g3 (6th trace). First MP1 is expressed (1st oblique arrow), then a switch is made to a partial form of MP2 (2nd oblique arrow). Note the approximately constant latency of g3–g4 coordination as the period increase in MP2, and the approximately constant phase of g4–g5 coordination. Following MP2 there is a tonic period, and then MP1 resumes. Calibration: vertical 1st trace 20 mV, 2nd trace 50 mV.

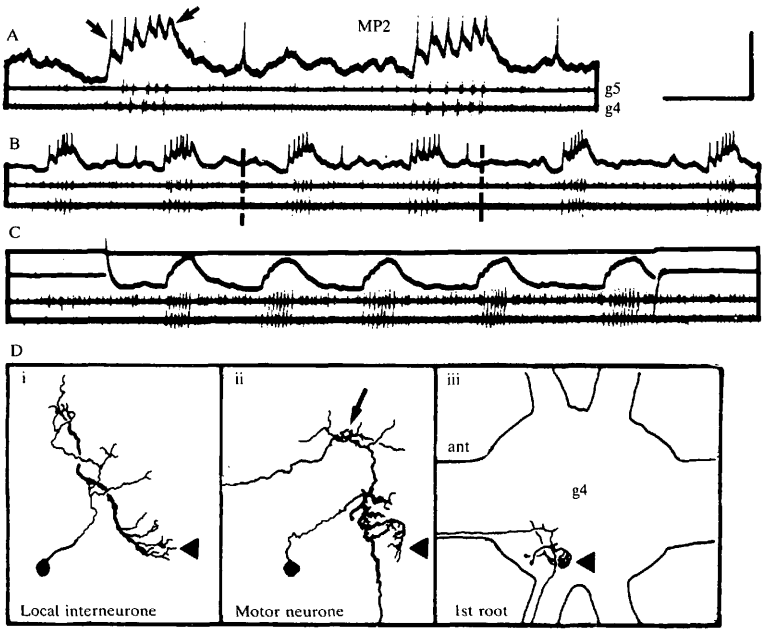

Fig. 12. (A)–(C) A g4 neurone, probably a motor neurone, which appears to be essential for the production of 'bursty' MP2. (A),(B) Large unitary EPSPs impinge on the neurone (1st trace), which are approximately synchronous with bursts of spikes recorded extracellularly from g5 (2nd trace) and g4 (3rd trace) within each cycle period. (A) shows the section of (B) between dotted vertical lines with an expanded timebase. Some EPSPs are suprathreshold (A: 1st arrow), and some are subthreshold (A: 2nd arrow). (C) Later in the preparation the intracellular recording deteriorated and the extracellularly recorded bursts ceased. Rhythmic activity was restored by injecting hyperpolarizing current (monitor in 1st trace, other traces the same, but displaced downwards). (D) *Camera lucida* drawings of Lucifer Yellow dye injection. A local interneurone (i) and a motor neurone (ii) are separately drawn to scale, and a sketch of their location within the ganglion is shown (iii). Filled triangles indicate a region of apparent contact, arrow in (ii) indicates a region where the precise anatomy is unclear. The penetrated neurone was probably the motor neurone: see text for discussion; ant, anterior. Calibration: vertical A 15 mV, B,C 60 mV; horizontal A 0·4 s, B 0·8 s, D(i,ii) 150 μm.

In another preparation simultaneous intracellular recordings were made in g4 from an anterior and posterior branch motor neurone during spontaneous switching between MP1 and 2 (Fig. 11). MP2 was fully expressed in g3 and 4, while g5 showed the period of MP2, but the phase and amplitude of MP1. The posterior branch motor neurone again received the sinusoidal and episodic sawtooth input, while the anterior branch motor neurone received an input which was approximately the 'mirror image' of the posterior branch. Thus during MP2 the anterior

branch motor neurone showed an extended period of depolarization, interrupted by periodic inhibition coincident with the ramp depolarization of the posterior branch motor neurone. The input to both motor neurones was subthreshold in MP1, but a few spikes were induced in the anterior branch motor neurone during MP2. A brief tonic period followed MP2 before the resumption of MP1.

To what extent can the observed waveforms of motor neurone input explain the characteristics of the motor programmes? The sinusoidal input fits very well with MP1. Indeed, the CPG was modelled as a sinusoidal oscillator on the basis of extracellular recordings during MP1 before any direct evidence of the synaptic mechanisms was available (Davis, 1969). The episodic sawtooth input appears adequate to explain several of the characteristics of MP2, including the shift in relative duration of posterior and anterior branch activity, the increased amplitude of output and the change of frequency. To determine whether it explains the change in interganglionic coordination requires simultaneous penetrations of neurones in different ganglia, which has not yet been attempted. However, the episodic sawtooth input does not explain the 'bursty' output often apparent within individual cycles in MP2. Some other form of synaptic input must be driving that aspect of the motor programme.

In one preparation MP2 was the only form of rhythmic output obtained, and the extracellular recordings from g4 and 5 displayed the 'bursty' type of output exclusively, without the more homogeneous background (Fig. 12). The bursts were coordinated in the two ganglia, with individual bursts in g4 preceding those in g5. The interganglionic latency recorded by extracellular electrodes was short, being about 20 ms for the initial bursts at the start of each cycle, and declining to only 5 ms by the end of the cycle. An intracellular recording was made from a g4 neurone which received large, highly phasic bursts of EPSPs during the power stroke phase of the rhythm (Fig. 12). This input was often suprathreshold. There was no sign of the episodic ramp type of input. The EPSPs were approximately synchronous with several large motor units recorded extracellularly which expressed the 'bursty' output, and which presumably were receiving the same or similar phasic excitatory input. During each cycle period the EPSPs summated, so that spikes towards the end of the cycle were induced earlier on the rising phase of the synaptic input. A similar effect occurring in each of the motor neurones causing the output might explain the slight decrease in latency within each cycle.

About 30 min after first penetrating the neurone, the membrane potential depolarized and the quality of the intracellular recording deteriorated. This was presumably due to damage to the neurone. As this happened, the synaptic input failed, and the rhythmic motor output from both g4 and g5 ceased (Fig. 12C). This implies that there was some feedback path from the neurone to the CPG such that normal activity in the neurone was necessary for the CPG function. Hyperpolarizing current injected into the neurone 'rescued' the recording, and restored both the synaptic input (now subthreshold) onto itself and the extracellularly recorded motor output from g4 and 5 (Fig. 12C).

Subsequent intracellular staining revealed a motor neurone with an axon in the anterior branch of the 1st root, an axon in the anterior connective which was either a collateral of the motor neurone or a dye-coupled interganglionic interneurone, and

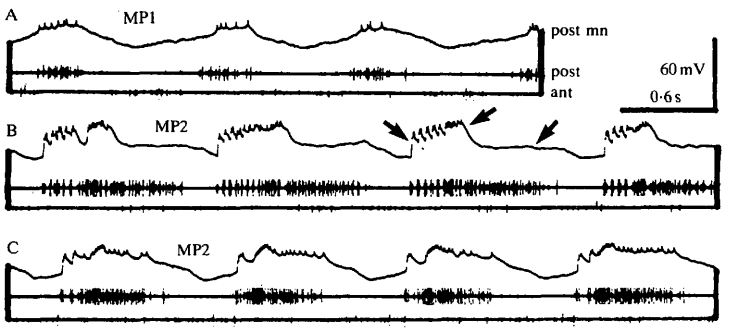

Fig. 13. (A)–(C) Three types of synaptic input, and two motor programmes, impinging on a single g4 posterior branch motor neurone. (A) During MP1 the motor neurone (1st trace) receives approximately sinusoidal suprathreshold input in phase with g4 posterior branch bursts (2nd trace). There is little activity in the g4 anterior branch (3rd trace). (B) During MP2 the motor neurone receives a series of large, apparently unitary suprathreshold EPSPs which are synchronous with extracellularly recorded bursts from several g4 posterior branch motor neurones (1st arrow). These appear to summate later in the cycle period to produce a sustained depolarization (2nd arrow), followed by a subthreshold depolarizing 'shoulder' (3rd arrow). (C) In some cycles the shoulder on the falling phase is also suprathreshold.

an interneurone local to the ganglion. The dye coupling suggests that these neurones might all have been electrically coupled. The recorded unit was tentatively identified as the motor neurone because this was the most brightly stained structure. Spikes recorded intracellularly could not be correlated with extracellularly recorded spikes, but the extracellular recording from g4 was rather noisy, and small spikes could have been obscured. Whatever its identity, the neurone recorded intracellularly was intimately involved in the circuitry producing MP2, since an adequate membrane potential was essential for the production of MP2 in both its own and an adjacent ganglion. If it was indeed the motor neurone this means that some motor neurones are involved in the production of MP2, just as some are involved in the production of MP1 (Heitler, 1978).

The large EPSPs recorded in the neurone described above are adequate to explain the 'bursty' characteristics of MP2, but are clearly different from the episodic sawtooth input described previously. Are there two pools of motor neurones receiving exclusively different input during MP2? One pool might receive episodic ramp input and produce the homogeneous background activity, while the other receives phasic EPSPs and produces the 'bursty' type of output. However, recordings from a g4 posterior branch motor neurone which spiked during MP1 and 2 showed three types of synaptic input (Fig. 13). During MP1 the motor neurone received approximately sinusoidal input (Fig. 13A). During MP2 the motor neurone received two distinct types of input: large EPSPs followed by a depolarizing shoulder. The EPSPs were synchronous with the characteristic 'bursty' spikes recorded extracellularly. They increased in frequency within each cycle, eventually

summating to produce a continuous depolarization. This ceased about mid-way through the cycle, and was followed by a shoulder of depolarization which coincided with a sustained homogeneous burst of spikes recorded extracellularly (Fig. 13B). In other cycles the motor neurone received only one or two phasic potentials early in the cycle, followed again by a depolarizing shoulder, which on these occasions was suprathreshold (Fig. 13C). This shoulder appeared similar to the falling phase of the episodic sawtooth impinging on other motor neurones. The shift in input waveform was reflected in the extracellular recordings, which were 'bursty' in the initial part of the cycle, and then became more homogeneous. Thus in this case a single motor neurone received three different forms of synaptic input, each of which was capable of inducing spikes, and was active in both motor programmes.

DISCUSSION

This study has two main conclusions: firstly, that the swimmerets can be driven by more than one motor programme; secondly, that switching between motor programmes can be induced by current injected into any one of several different neurones. The major problems have been the rather rare and unpredictable occurrence of the phenomena under investigation, and the low probability of penetrating the appropriate neurones with microelectrodes. Does the consequent small sample size invalidate either of the main conclusions?

Do different motor programmes occur?

Three possible objections may be raised to the first conclusion. The first is that MP2 does not really exist, but is merely a disrupted form of MP1. MP1 is normally very regular and constant, and consequently deviations from it are easily recognized. Such deviations undoubtedly occur. Some are irregular and unpatterned (Fig. 2), but others take a constant form, which is repeated many times within and between preparations (Fig. 3). These may legitimately be classed as separate motor programmes. There is some doubt as to whether MP2 is a single motor programme, or several similar programmes, but there is no doubt that it is separate and distinct from MP1.

The second objection is that MP2 is an artefact resulting from damage or deterioration of the preparation. It is difficult to define objective criteria for the 'health' of an isolated abdominal nerve cord, but MP2 has been observed in extracellular recordings prior to making any intracellular recordings, so it cannot result from damage caused by microelectrode penetration. It is unlikely to result from deterioration of the preparation since it has been observed at the start of experiments in which preparations have remained active for several hours. It is hard to discount the possibility that the dissection itself might somehow induce damage. However, although no systematic study of behaviour in the intact animal has been made, vigorous swimmeret beating with MP2-type coordination (i.e. anterior-to-posterior) *has* been observed in the undissected, undamaged animal (K. Sillar, personal communication).

The third objection is that since MP2 has only been observed in about 7% of

preparations, it is too rare to be significant, and perhaps arises from a mutation or developmental aberration. Two points argue against this. First, the crayfish have not been reared in a closed colony, but under open conditions. Thus there will have been little inbreeding, and selective pressures will have remained normal (c.f. Pearson & Goodman, 1979). Second, there is no reason to expect the preparation to run the gamut of its behavioural repertoire in relatively invariable experimental conditions. In the lobster, different swimmeret motor programmes are associated with different types of behaviour of the whole animal (Cattaert & Clarac, 1983). It may be that MP2 in the crayfish is normally associated with a behaviour which is rarely expressed under the experimental conditions used in this study.

Can motor programme switching be induced?

Three objections may also be raised to the second main conclusion. The first is that the motor programme switching which follows current injection into single neurones is not caused by it, but merely coincidental. Although the sample size is small in terms of the number of neurones displaying putative switch-inducing capability (only four have been recorded – three are described above, the fourth was like that of Figs 4–7 except that discrete PSPs were visible), the success rate for individual neurones is high. Sixteen out of 19 trials induced switching, with 100 % success (six trials) for the 'best' preparation. Unfortunately, continuous records were not kept for the duration of these experiments, so no probability analysis can be attempted. However, in most preparations spontaneous bouts of MP2 were relatively irregular and infrequent, and thus it is very unlikely that the 'induced' bouts were coincidental.

The second objection is that switch-induction is spurious, resulting from damage (perhaps due to microelectrode penetration) to a neurone intimately involved in the CPG. Against this it is argued that there was no difference in the motor programme switching recorded extracellularly before and after microelectrode penetration. Furthermore, the membrane potentials of the recorded neurones were normal (50–70 mV), and stable for the duration of the experiment. In one preparation where damage is thought to have occurred (Fig. 12), the resulting membrane potential depolarization and decline in spike and EPSP amplitude were obvious.

The third objection is that the results are special cases which do not reflect the normal functioning of these neurones. This could be countered if the putative switch-inducing neurones had been positively identified and repeatedly penetrated in several preparations, but unfortunately, owing to the constraints of the experimental system, this is not the case. It thus remains an open question as to whether these neurones *always* induce switching, or whether the preparation has to be in a particular state. However, there is no evidence to suggest that this state, if it exists, is in any way abnormal.

Control of motor programmes

Three questions were initially posed in this project: first, what is the nature of the mechanisms generating the different programmes; second, are the same motor neurones involved in each programme; and third, how is switching between

programmes accomplished. If the two major conclusions are accepted, partial answers to these questions can now be attempted.

Mechanism of generation: theoretical basis

One of the first steps in analysing the mechanism of rhythm generation is to determine which neurones are components of the CPG. Two criteria must be met to establish a neurone in this role. First, the neurone must itself display rhythmic activity in the form of membrane potential oscillations or spike bursts which are phase-locked to the rhythm in question. Second, altering the normal timing of activity of the neurone should permanently reset the activity of all other neurones participating in the rhythm. This second criterion is of course graded; it leaves open the question of how strongly the 'normal timing' has to be altered to achieve resetting. This depends on the strength of the connections between the neurone in question and the rest of the CPG. The CPG should not be regarded as a uniform, discrete module with clearly defined limits, but rather as a more diffuse network in which some elements are of greater importance than others.

How does motor programme switching fit with the concept of a CPG? The mechanisms may fall into three simple categories. The first (type 1) mechanism is that there is a single ensemble of neurones, whose network interactions are such as to produce different activity in its individual constituents. In such an ensemble all the constituents are active all the time, but selective gating ensures that only some are effective in producing motor output. Switching is achieved by changes in gating between the motor output pathways and the CPG constituents. The second (type 2) mechanism is that there is a single ensemble of neurones which itself can be switched to produce different forms of output. It is not difficult to construct a hypothetical network whose output can be switched between different states by perturbing the activity of one or more of its members. Thus the connections between the CPG and the output pathways are not modulated as in type 1, but rather the activity of the CPG itself is changed. The third (type 3) mechanism is that there are separate ensembles of neurones with different properties, each connected to the appropriate output pathways, but only one of which is active at any given time.

These possibilities are not mutually exclusive. Partially overlapping networks (type 2+3) can be envisaged, with some neural elements participating in all of the motor programmes, and some in only one. The scaphognathite rhythm of crabs (Simmers & Bush, 1983*b*) appears to result from a type 1+2 mechanism. The scaphognathites beat rhythmically to drive water across the gills, and periodically reverse the direction of flow (Arudpragasam & Naylor, 1966). Different subsets of motor neurones are active in forward and reverse beating (Young, 1975), and receive input in different phases of what appears to be a single CPG (Simmers & Bush, 1983*a*). The input impinges on all the motor neurones all the time, but is masked in the inactive subset by superimposed tonic inhibition (Simmers & Bush, 1983*b*). Thus there is a single CPG with multiphasic output which is continuously active (type 1). However, the CPG itself changes output frequency (although not waveform) in the different mode, suggesting a type 2 component to the switch.

The idea of there being a single CPG for each segmental appendage which can

somehow generate different motor programmes has the appeal of conceptual simplicity and evolutionary parsimony. It fits with the notion of crustacean limbs having all evolved from primitive appendages which combined ventilatory, locomotory and feeding functions with minimal serial differentiation (Manton, 1977). However, even in very primitive crustaceans there is evidence for limbs being driven by more than one CPG. The syncarid *Anaspides* uses its thoracic endopodites for locomotion and exopodites for ventilation. These structures are thus rami of the same segmental appendage, and yet they show 'considerable independence' in their coordination (Macmillan, Silvey & Wilson, 1981). Similarly the swimming exopodite and ventilatory epipodite on the first thoracic limb of the mysid shrimp *Praunus* may maintain different cycle periods which can be varied independently, with little tendency for phase coordination (Laverack, Neil & Robertson, 1977).

Mechanism of generation: experimental basis

Motor-driver interneurones which are integral to the CPG of MP1 (Figs 4–8) have considerably reduced and altered membrane potential oscillations during expression of MP2. The type 1 mechanism for generating multiple motor programmes (a single, continuously active CPG with differential output) can thus be eliminated. Furthermore, the reduction in membrane potential oscillation during MP2 suggests that these interneurones participate only weakly, if at all, in generating MP2 (cf. the first criterion for CPG participation described above). This implies that a type 3 mechanism is, at least in part, responsible for switching.

A type 3 mechanism is further supported by intra- and extracellular recordings of motor activity. During MP1 most motor neurones receive approximately sinusoidal input in which the underlying waveform appears relatively smooth. During MP2 the input can be in either or both of two forms: an episodic sawtooth input, and/or large unitary EPSPs. This reflects the two sorts of output recorded extracellularly; an homogeneous background presumably resulting from the sawtooth input, and 'bursty' spikes clearly resulting from the unitary EPSPs. The sawtooth input of MP2 could conceivably result from a modulation of the circuitry producing the sinusoidal MP1 input, i.e. a type 2 mechanism, but the unitary EPSPs of MP2 appear to derive from a totally different source. The synchronous output of several motor neurones during bursty activity suggests that they are driven by a relatively small number of closely coupled spiking interneurones. The large unitary EPSPs have never been seen in any motor neurone during the production of MP1, suggesting that the interneurones responsible for their production are only active in MP2; i.e. a type 3 mechanism.

During MP1, interganglionic coordination is phase constant, and thus the metachronal latency varies with cycle period and cannot be explained simply in terms of conduction velocity. The interneurones mediating interganglionic coordination must be connected to the CPGs so as to vary the timing of their activity with cycle period. During MP2 on the other hand, the metachronal delay is much more constant, even though the cycle period is more variable than MP1. The bursty activity may be nearly synchronous in adjacent ganglia, with a short delay consistent with conduction velocity. This suggests that it is driven either by spiking inter-

ganglionic interneurones, or by elements closely coupled to them. The anterior-to-posterior coordination of the bursty activity shows that the interneurones must be conducting caudally. Thus the bursty activity of MP2 has a very different co-ordination mode to the activity of MP1, which further supports the hypothesis that it derives from a different CPG. The coordination mode of the homogeneous activity of MP2 is less clear, and it will require intracellular recordings from neurones in adjacent ganglia to investigate this.

Involvement of motor neurones

Extracellular recordings clearly show that more motor neurones are active at higher frequency during MP2 than MP1. Only a few intracellular recordings have been made from motor neurones during switching between the two programmes, but in each case these have shown that the motor neurones receive the appropriate form of synaptic input in *both* programmes. Indeed, recordings from one motor neurone revealed all three types of synaptic input (Fig. 13). Each type was suprathreshold, but that occurring during MP2 was of greater amplitude and induced more spikes than that during MP1. Thus the recruitment of motor neurones during MP2 could simply be the result of the greater amplitude of input. Although the available data are limited, there is no evidence for particular motor neurone pools being selectively 'tuned in' to the different CPGs. This is not to suggest that *all* motor neurones receive the same input in the two programmes; Fig. 10 clearly shows spikes recorded extracellularly *before* the depolarization recorded intracellularly.

Some swimmeret motor neurones have previously been shown to be intimately involved in the CPG for MP1 (Heitler, 1978). One such motor neurone received suprathreshold sinusoidal input during MP1, and suprathreshold episodic sawtooth input during MP2. Thus a member of the CPG for one motor programme remained active but changed its waveform during the other. This is consistent with a type 2 mechanism, in which a single ensemble of neurones alters its activity. However, in order to demonstrate resetting in MP1 it was necessary to induce spiking at a considerably higher frequency than that which occurred spontaneously. Unfortunately, the ability of the motor neurone to reset the phase of MP2 as well as MP1 was not tested. In another preparation a neurone was recorded which appeared to be an essential part of the CPG for MP2, in that damage-induced depolarization caused inhibition of MP2 which could be reversed by injecting hyperpolarizing current. This neurone was tentatively identified as an anterior branch motor neurone, which, if correct, indicates that some motor neurones are intimately involved in the CPG for MP2 as in that for MP1.

Motor programme switching

At the motor neurone level switching is achieved by a change in the characteristics of the synaptic input. This switch, which can affect the output of some hundreds of neurones in at least three ganglia, can be experimentally induced by injecting small amounts of current into any one of at least three distinct types of interneurones. Thus whatever the mechanism underlying switching, the factors

determining which particular motor programme is expressed at any given time must be finely balanced. In each case, switching was induced by injecting current of a polarity such as to *reduce* power stroke activity and/or *increase* return stroke activity. Although few data are available, the tentative conclusion is that locking MP1 into its return stroke phase is the crucial step in initiating MP2.

The total number of neurones capable of inducing switching is not known, but four different interneurones with this property have been encountered in one hemiganglion of g4. The majority of interneurones, however, even amongst those involved in the generation of the motor programmes, do *not* induce switching when injected with current. It is not known whether spontaneous bouts of MP2 occurring in the isolated abdominal CNS are accompanied by *simultaneous* changes in the waveforms of all the interneurones capable of inducing switching (caused by some switching input from a higher centre impinging on them all), or whether these changes are triggered sequentially through a single particular interneurone. However, the experimental evidence indicating the existence of a limited number of such crucial points within the CPG suggests that these would form suitable targets for motor programme control elements in the intact animal.

Early results in this project were obtained while I was working in the laboratory of Professor B. Mulloney, for whose encouragement and financial support (USPHS grant) I am grateful. Further data were obtained with the help of grants to the author from the Royal Society and the Science and Engineering Research Council of the U.K. A preliminary report of some data has been published as conference proceedings (Heitler, 1981).

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