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BRAIN OSCILLATOR(S) UNDERLYING RHYTHMIC CEREBRAL AND BUCCAL MOTOR OUTPUT IN THE MOLLUSC, *PLEUROBRANCHAEA CALIFORNICA*

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SUMMARY

1. Tonic (d.c.) intracellular depolarization of the previously identified phasic paracerebral feeding command interneurones (PCps) in the brain of the carnivorous gastropod *Pleurobranchaea* causes oscillatory neural activity in the brain, both before and after transecting the cerebrobuccal connectives. Therefore, cycle-by-cycle ascending input from the buccal ganglion is not essential to cyclic brain activity. Instead the brain contains an independent neural oscillator(s), in addition to the oscillator(s) demonstrated previously in the buccal ganglion (Davis *et al.* 1973).

2. Transection of the cerebrobuccal connectives immediately reduces the previously demonstrated (Kovac, Davis, Matera & Croll, 1983) long-latency polysynaptic excitation of the PCps by the polysynaptic excitors (PSEs) of the PCps. Therefore polysynaptic excitation of the PCps by the PSEs is mediated by an ascending neurone(s) from the buccal ganglion.

3. The capacity of feeding command interneurones to induce neural oscillation in the isolated brain declines to near zero within 1 h after transection of the cerebrobuccal connectives, suggesting that this capacity is normally maintained by ascending information from the buccal ganglion.

4. The results show that this motor system conforms to a widely applicable general model of the neural control of rhythmic behaviour, by which independent neural oscillators distributed widely in the central nervous system are coupled together to produce coordinated movement.

INTRODUCTION

Rhythmic movements can in principle originate by one of two different neural mechanisms. First, the underlying rhythm may be generated by a single 'master' neural oscillator, the output of which is then imposed upon follower neurones. Second, the rhythm may be generated simultaneously by several independent neural oscillators, which are coupled together to produce coordinated movements. The second mechanism, i.e. multiple, coupled oscillators, has been documented for

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several motor systems in both invertebrates (for reviews see Delcomyn, 1980; Kennedy & Davis, 1977) and vertebrates (Grillner, 1975, 1981). Recently, however, it has been proposed that the buccal motor rhythm(s) of the mollusc *Pleurobranchaea* is generated by a single oscillator located only in the buccal ganglion (Cohan & Mpitsos, 1983). This interpretation stands in contrast to our earlier deduction that the buccal motor system of *Pleurobranchaea* contains independent neural oscillators in the brain and buccal ganglion (Davis, Siegler & Mpitsos, 1973).

The question raised by the interpretations of Cohan & Mpitsos require resolution for two reasons. First, if true, the buccal motor system of *Pleurobranchaea* would represent an exception to an apparently widespread rule of the central organization of motor systems. Second, our continuing studies of the neural mechanisms of associative learning in this motor system (Davis *et al.* 1974, 1983; Davis & Gillette, 1978; Davis, 1983) depend upon accurate background information on the underlying neural organization of the buccal motor system. Accordingly, we have tested the hypothesis proposed by Cohan & Mpitsos. Our results demonstrate the existence of an independent neural oscillator(s) in the brain, which is normally coupled to the buccal oscillator(s). Our study therefore shows that this motor system operates by the coupling of independent, distributed neural oscillators. The results also provide evidence regarding the neural mechanisms by which these independent neural oscillators are coupled.

MATERIALS AND METHODS

Experiments were performed on the carnivorous marine gastropod mollusc *Pleurobranchaea californica*. Specimens were obtained by trawling in Monterey Bay at depths of 60–90 m using the UCSC research vessel *Scammon*. Prior to experiments animals were maintained in separate plastic containers in fresh, running sea water at ambient temperatures (11–17 °C) at UCSC's Long Marine Laboratories and fed raw squid on a weekly schedule. Animals used for experiments were 70–300 ml in volume.

Neurophysiological experiments were performed on the isolated central nervous system (CNS), consisting of the brain (cerebropleural ganglion) and buccal ganglion, connected by the paired cerebrobuccal connectives (CBCs). All other ganglia were removed prior to experiments. Preparations were pinned and/or glued with Super-Glue (Duro Corp.) to the Sylgard (Dow Chemical) floor of an experimental chamber in cold (12 ± 1 °C) sea water. The brain was manually desheathed using fine forceps. Glass capillary microelectrodes (tip resistance, approximately 10 MΩ measured in sea water) were inserted under visual control into previously identified feeding command interneurones in the brain, including the phasic paracerebral command interneurones (PCps) and the polysynaptic excitors (PSEs) of the PCps (Kovac, Davis, Matera & Gillette, 1982; Kovac *et al.* 1983). These feeding command interneurones were identified using criteria developed previously (*ibid.*), including position of the soma in the brain, presence of a characteristic cyclic inhibition, presence of a descending axon in the ipsilateral CBC, and the capacity to elicit cyclic motor output from efferent nerves that innervate buccal muscles. Studies on whole animal preparations have shown that tonic depolarization of these command interneurones induces ingestion, rather than egestion, and that these neurones are active during ingestion but sil[illegible]

ing egestion (Gillette, Kovac & Davis, 1978, 1982). The identification of the motor programmes as those that normally underlie ingestion (feeding) rather than egestion was accomplished using several qualitative and quantitative criteria developed previously (Croll & Davis, 1981, 1982; R. P. Croll, M. P. Kovac & W. J. Davis, in preparation; R. P. Croll, M. P. Kovac, W. J. Davis & E. M. Matera, in preparation).

Extracellular recordings were made with polyethylene or glass capillary suction electrodes from two nerves of the buccal ganglion, namely the radula retractor nerve or root 1 (r1) and the radula retractor nerve or root 3 (r3). Extracellular recordings were also routinely made from three brain nerves, the mouth nerve (MN), the tentacle nerve (TN) and the small oral veil nerve (SOVN) (Davis *et al.* 1973). Recordings were amplified and displayed on a Tektronix oscilloscope for filming with a 70 mm oscilloscope camera, and/or recorded on a Brush-Gould eight-channel pen recorder. In order to uncouple the brain and buccal ganglion, the CBCs were cut with fine dissecting scissors, usually near the buccal ganglion, but also in the middle and in some cases at their exit from the brain.

RESULTS

In several previous papers it has been reported that tonic depolarization of single feeding command interneurones (PCps or PSEs) in the isolated CNS preparation causes cyclic motor output (e.g. Gillette *et al.* 1978, 1982; Kovac *et al.* 1982, 1983). This motor output has been shown to represent the ingestion (feeding) motor programme (R. P. Croll, M. P. Kovac, W. J. Davis & E. M. Matera, in preparation). The same result was obtained routinely in the present work on the isolated CNS preparation. Injection of tonic (d.c.) current into the soma of a PSE and/or a PCp caused cyclic bursts of action potentials in these command interneurones and simultaneous rhythmical, coordinated extracellular bursts in three efferent nerves of the brain and buccal ganglion (e.g. Fig. 1).

In order to test the hypothesis that the brain contains neural oscillator(s) independent of those demonstrated previously in the buccal ganglion, experiments similar to that shown in Fig. 1 were performed on the isolated brain. Fig. 2 shows an experiment in which four command interneurones were simultaneously penetrated and stimulated. Records from only three command interneurones are shown: the fourth was monitored with an audio system. Immediately (2 min) prior to the recording shown in Fig. 2A, both CBCs were cut. The sudden and large synaptic input to the brain caused by severing these central connectives induced sustained cyclic activity in the command interneurones and in the efferent nerves (Fig. 2A). The production of this cyclic activity does not alone constitute rigorous evidence for a separate brain oscillator(s), however, since activation of CBC oscillatory properties (Cohan & Mpitsos, 1983) by injury currents could in principle underlie the rhythmic brain activity observed immediately after cutting the CBCs (Fig. 2A).

Over a period of minutes, the cyclic brain activity induced by CBC transection gradually declined. During this time the frequency of the cyclic rhythm decreased from the initial value of about 0·1 Hz to less than 0·003 Hz (Fig. 2B, C). When the ivity induced by cutting the CBCs had fully subsided (Fig. 3A), tonic (d.c.)

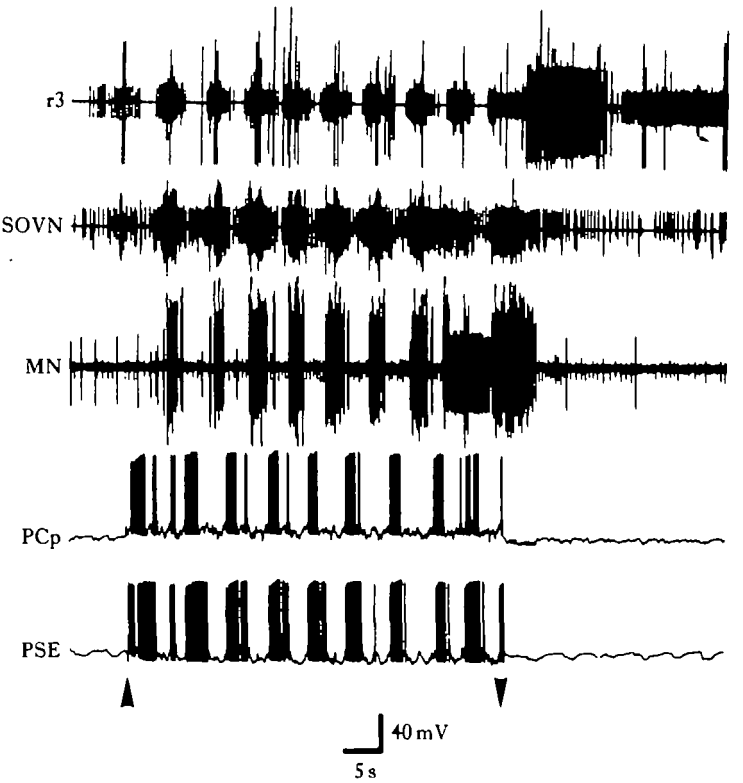

Fig. 1. Effects of injecting tonic (d.c.) current (between arrows) into the somata of two ipsilateral feeding command interneurones (PCp and PSE) in the brain of *Pleurobranchaea* before cutting the cerebrobuccal connectives (CBCs). Tonic stimulation of the command neurones induces cyclic bursting in the command interneurones and in feeding nerves of the buccal ganglion (r3) and brain. Abbreviations: r3, root 3 of the buccal ganglion; SOVN, small oral veil nerve of the brain; MN, mouth nerve of the brain.

current was injected into the somata of all four impaled command interneurones simultaneously. Such stimulation of the command interneurones in the now isolated and quiescent brain immediately induced vigorous, cyclic efferent activity in the SOVN and MN, and coupled cyclic activity in the command interneurones themselves (Fig. 3B). The frequency of this induced oscillation was approximately 0·1 Hz.

This rhythmic neural activity persisted for as long as the stimulus was maintained and stopped immediately upon cessation of the stimulus (Fig. 3C, D). The frequency of the rhythm could be more than doubled (>0·2 Hz) by increasing the amount of current injected into the four impaled command interneurones (not shown). Therefore this cyclic neural activity in command neurones and efferent nerves was presumably causally related to the injection of current into the command interneurones. Induction of such rhythmic neural activity in the isolated brain occurred even when the CBCs were transected at their exit from the brain, indicating that the underlying neural oscillator was located within the brain.

A similar experiment on a different preparation is quantitatively illustrated in Fig. 4. Here two feeding command interneurones, a PSE and an ipsilateral PCp, were stimulated together and separately while motor activity was recorded from ipsilate

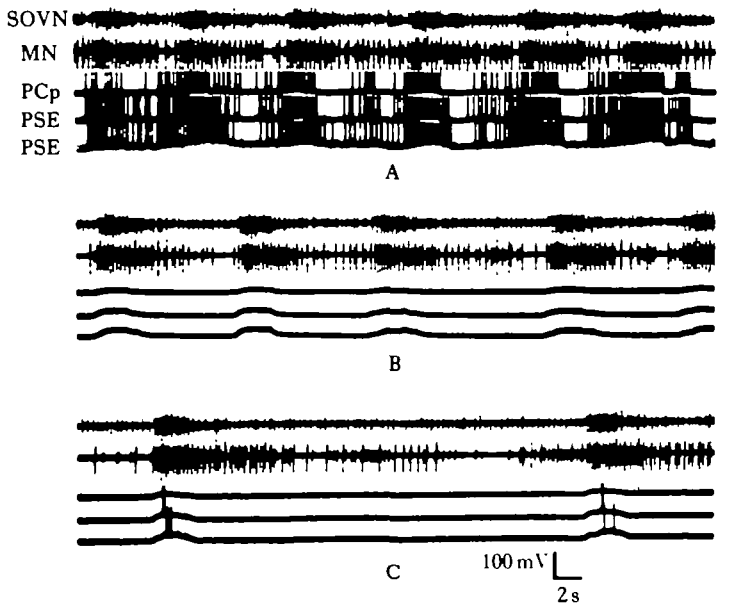

Fig. 2. Neural activity in brain nerves and feeding command interneurones in the brain immediately following transection of both cerebrobuccal connectives in the same preparation as in Fig. 1. (A) Vigorous cyclic activity recorded 2 min following the cut. The command neurones were weakly depolarized throughout the record by injecting current into their somata. The record from the contralateral PCp (bottom trace in Fig. 1) is not shown although the cell was still impaled and confirmed to be active during the record. (B) Cyclic activity recorded 7 s after A above with no depolarization of the command interneurones. (C) Cyclic activity recorded 2 min and 15 s and after B above. Abbreviations as in Fig. 1.

brain nerves (MN, TN) and nerves of the buccal ganglion (r1 and r3). Only the activity of the MN is presented in Fig. 4 as an index of rhythmic motor output. Tonic stimulation of either the PSE or the PCp alone or together prior to transecting the CBCs caused rhythmic efferent output in the MN (Fig. 4) and in the other three recorded nerves (not shown). As documented in earlier work (Kovac *et al.* 1983; R. P. Croll, M. P. Kovac, W. J. Davis & E. M. Matera, in preparation), the PSE was more efficacious than the PCp in eliciting rhythmic motor output (Fig. 4). Cutting the CBCs induced vigorous cyclic activity in the MN (Fig. 4) and also in the two impaled command interneurones and in the other three efferent nerves (not shown). As in the preceding experiment described (Fig. 3), this induced rhythmicity gradually subsided over the course of a few minutes (Fig. 4). Simultaneous intracellular stimulation of the two command interneurones in the now isolated and quiescent brain again induced cyclic motor output in the MN, although the frequency of the rhythm was lower than prior to cutting the CBCs (Fig. 4). As expected, cyclic activity in buccal nerves was not elicited by stimulation of command interneurones in the brain after cutting the CBCs.

The experiment illustrated in Figs 3 and 4 was performed on seven preparations. Without exception, tonic intracellular stimulation of one or more feeding command interneurones in the brain before cutting the CBCs induced vigorous rhythmic activity of brain nerves (SOVN and MN), and coupled bursting in the command neurones themselves, as exemplified in Fig. 1. Following transection of the two CBCs, tonic

depolarization of one or more brain command interneurones caused cyclic burst[illegible] in the command interneurones themselves in all seven preparations. This central neural oscillation was accompanied by rhythmic discharge recorded extracellularly from peripheral brain nerves (MN, SOVN, TN) in three of the seven preparations, as exemplified in Figs 3 and 4. In the remaining four preparations, coupled cyclic extracellular activity in the brain nerves was absent or at least much less obvious immediately after the brain was isolated by transecting the CBCs (e.g. Fig. 5). These results demonstrate that the brain contains a neural oscillator(s) capable of producing cyclic activity in the feeding command interneurones, and that this oscillator(s) can operate without driving simultaneous cyclic activity in efferent brain nerves. It follows that the coupling of the brain oscillator with efferent neurones is variable, and may depend in part on input from the buccal ganglion. As discussed later, this variable coupling between the brain oscillator(s) and the motoneurones that it drives may partially explain the results of Cohan & Mpitsos (1983).

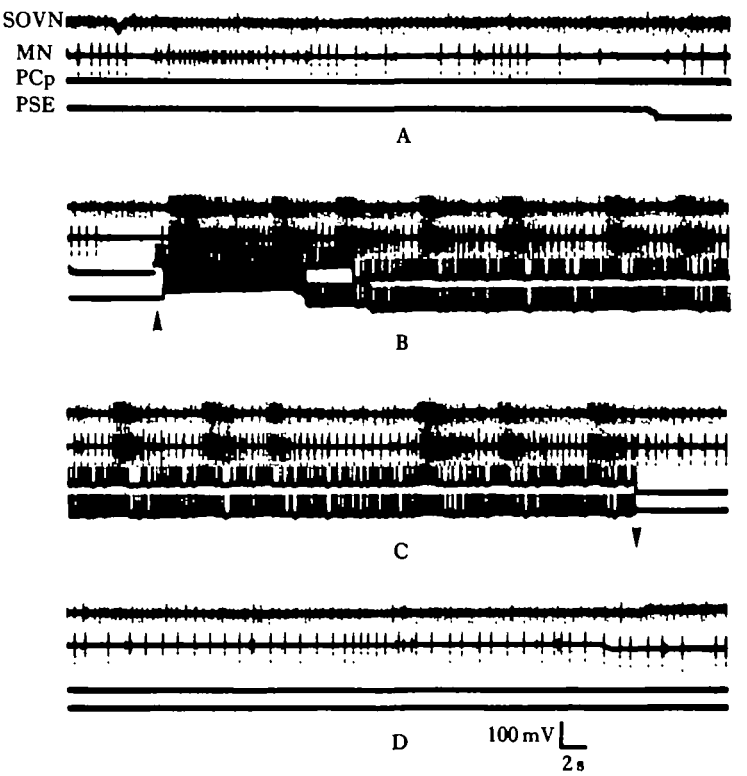

Fig. 3. Neural activity in brain nerves and feeding command interneurones in the brain in the same preparation as in Figs 1 and 2, recording beginning 1 min after the record in Fig. 2C. (A) Lack of cyclic activity in absence of command interneurone stimulation. (B) Cyclic activity in the brain nerves and feeding command interneurones in response to current injection into the command interneurones (arrow near the beginning of the record). Records from contralateral command interneurones are not shown although these cells were still impaled and made to fire by current injection simultaneously with those shown. (C) Cessation of cyclic activity upon release of command interneurone depolarization (near the end of the record). (D) Return to relative quiescence following command interneurone depolarization. The four records (A–D) are continuous. Abbreviations as in Fig. 1.

A pronounced effect of removing the buccal ganglion was observed in the polysynaptic pathways documented earlier (Kovac *et al.* 1983) from the PSEs to the PCps. Previously it was shown that the PSEs send descending axons to the buccal ganglion *via* the ipsilateral CBC, and that the PSEs produce a characteristic, long-latency chemical

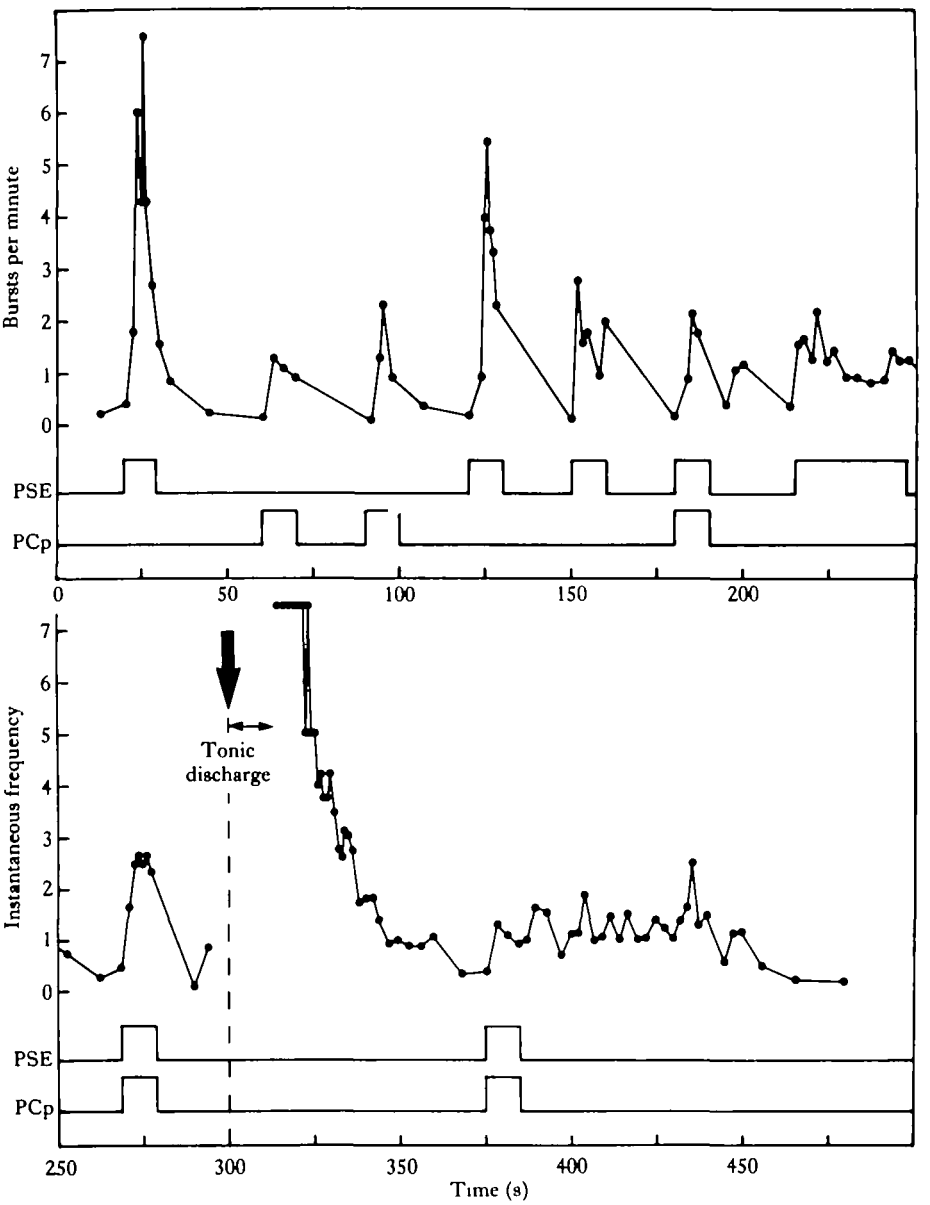

Fig. 4. Graphical representation of an experiment like that shown in Fig. 3, but from a different preparation. Intracellular stimulation of the feeding command interneurones (a PSE or polysynaptic excitor and a PCp or phasic paracerebral command neurone) is indicated on the lower two traces, while the instantaneous frequency of cyclic bursts of motor activity in the mouth nerve of the brain is indicated on the upper trace. The two sets of traces are continuous. The cerebrobuccal connective(s) (CBCs) were cut near the beginning of the lower set of traces (arrow).

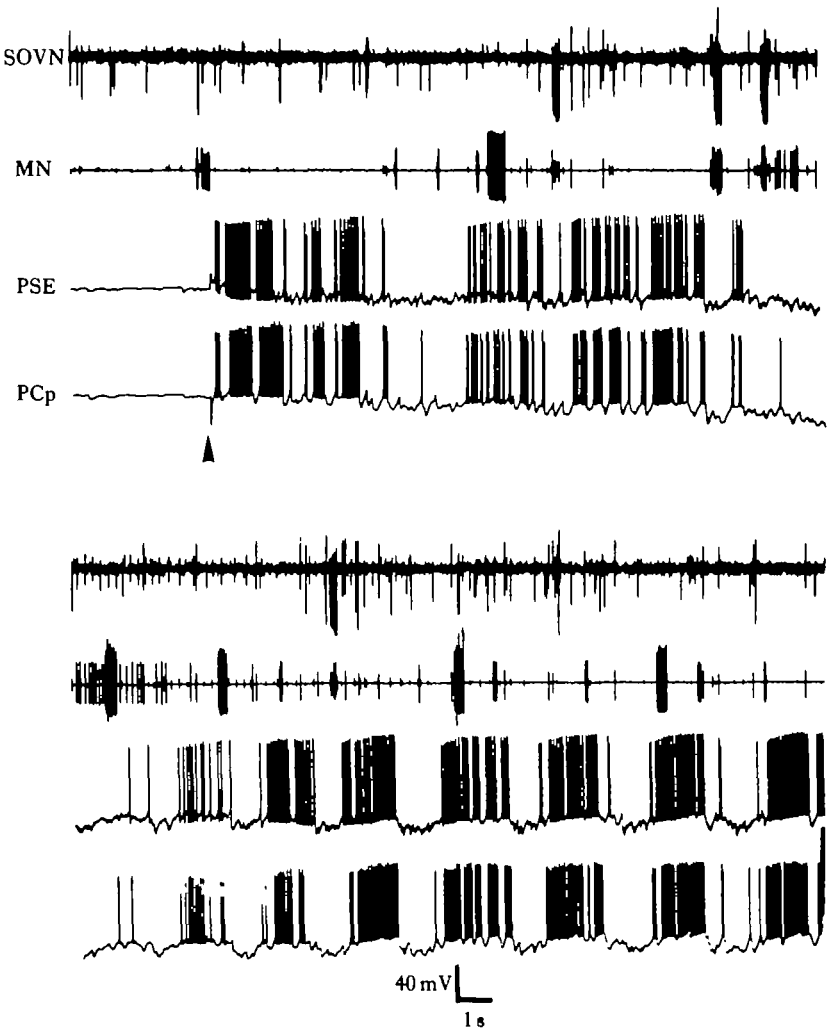

Fig. 5. Cyclic bursting induced by depolarizing the somata of two feeding command interneurones (a PSE and a PCp) after cutting both cerebrobuccal connectives. The beginning of current injection is indicated by the upward arrow. Extracellular activity of two brain nerves, the SOVN and MN did not show a clear relation with command cell bursting. The two sets of records are continuous.

polysynaptic excitation of the PCps (*ibid.*). In Fig. 6A, for example, the amplitude of this polysynaptic excitation was 25 mV before cutting the CBCs. Immediately upon isolating the brain by transection of the CBCs, however, this component of the PCp response to PSE stimulation declined to approximately 8 mV (Fig. 6B; note different voltage calibration from Fig. 6A). Even when the PSE was induced to fire at more than twice the discharge frequency than before CBC transection, this long-latency polysynaptic excitation was substantially reduced (50–75 %) in comparison with pre-transection values ($N = 4$ preparations). These findings show that a neurone(s) that ascends in the CBC from the buccal ganglion, i.e. one or more of the previously identified corollary discharge (CD) interneurones (Davis *et al.* 1973, 1974), mediates the major part of the long-latency polysynaptic excitation of the PCps by the PSE

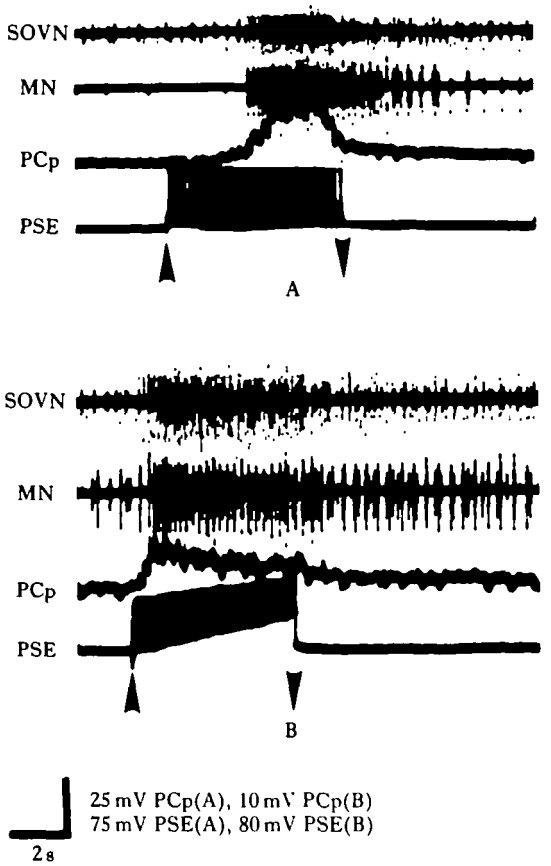

Fig. 6. Intracellular stimulation (between arrows) of a PSE before (A) and approximately 10 min after (B) cutting both cerebrobuccal connectives. Before cutting the CBCs (A) the PSE induces the characteristic large amplitude (25 mV) long-latency chemical polysynaptic excitation of the phasic paracerebral neurone (PCp). After cutting the CBCs (B) this component of the PCp response is greatly reduced (to ~8 mV) despite driving the PSE at a higher discharge frequency.

In all of the experiments reported above, the oscillatory activity of the brain was elicited within a few minutes (<30 min) of transecting the CBCs. In a separate series of seven experiments on seven different preparations, the CBCs were transected before the brain was removed from the animal, and feeding command interneurones were stimulated intracellularly from 1 to 3 h later. In none of these cases did tonic intracellular depolarization of the feeding command interneurone(s) in the brain cause clear cyclic output in feeding nerves of the brain. In only two of the seven experiments did tonic intracellular depolarization of the command interneurones induce these neurones to fire in their normal pattern of cyclic bursts, and in both of these cases the rhythmicity was weak. In contrast, when the brain and buccal ganglion are left connected, rhythmic motor output can be driven for hours by intracellular depolarization of the command interneurones (not shown). Negative findings are by nature inconclusive, but these results are consistent with the hypothesis that ascending information from the buccal ganglion contributes to the capacity of the brain oscillator(s) to generate cyclic neuronal activity.

DISCUSSION

The main conclusion of this paper is that there exists within the brain of *Pleurobranchaea* a neuronal oscillator(s) that is independent of the separate oscillator(s) demonstrated previously in the buccal ganglion (Davis *et al.* 1973). The normal function of these coupled oscillators is presumably to produce the cyclic motor output that underlies feeding behaviour in this mollusc. The evidence for this identity is as follows. First, previous studies on behaving whole animal preparations have shown that intracellular stimulation of the same command interneurones as stimulated here, namely the phasic paracerebral neurones or PCps, induces only feeding movements (Gillette *et al.* 1978, 1982). Second, these same command neurones are excited by food stimuli and are active cyclically in association with feeding movements in whole animal preparations, but are silent during egestion movements (Gillette *et al.* 1982, Fig. 8). Third, it has recently been shown that intracellular stimulation of the PCps elicits the identified ingestion motor programme or its characteristic components, but never the egestion motor programme and seldom its characteristic components (R. P. Croll, M. P. Kovac, W. J. Davis & E. M. Matera, in preparation). It has been suggested that the same neural oscillators that subserve feeding behaviour also subserve egestion movements (McClellan, 1982*a*,*b*; Croll & Davis, 1981, 1982; R. P. Croll, M. P. Kovac, W. J. Davis & E. M. Matera, in preparation), although independent command pathways for the two motor programmes have been documented (R. P. Croll, M. P. Kovac & W. J. Davis, in preparation; R. P. Croll, M. P. Kovac, W. J. Davis & E. M. Matera, in preparation).

The present experiments on the isolated CNS preparation have shown that cyclic motor output can be induced by stimulation of feeding command interneurones even when the CBCs are cut at their exit from the brain. Therefore this oscillation is generated within the confines of the brain, and does not depend upon interactions with neurones in the CBCs or other ganglia. Beyond this general localization of the brain oscillator(s), its nature is unknown. It is possible that the oscillation is produced entirely by neurones whose processes are normally restricted to the brain. Alternatively, or in addition, it is possible that the oscillation involves interactions with the terminal processes of ascending neurones from the buccal ganglion. Further experiments would be required to distinguish between these possibilities.

Comparison with previous studies

We proposed earlier that the buccal motor system of *Pleurobranchaea* operates by the coupling of independent neural oscillators located in the brain and buccal ganglion (Davis *et al.* 1973). This conclusion was based on the observation that cyclic efferent activity occurs in nerves of the isolated brain and the isolated buccal ganglion. Such motor rhythms occur spontaneously or can be induced by tonic extracellular stimulation of the appropriate nerves (*ibid.*). Cohan & Mpitsos (1983) confirmed these observations, but also reported that tonic electrical stimulation of the isolated CBCs caused cyclic output from this nerve, implying that neurones within the CBCs have oscillatory properties. They suggested that such properties could account for the observed oscillation of the isolated brain. Cohan & Mpitsos (1983) also found that rhythmic brain activity induced by tonic extracellular stimulation of a buccal nerve

stomatogastric nerve, ceased when ascending information in the CBCs was eliminated by sucrose gap or anodal block. From these observations Cohan & Mpitsos concluded that 'the rhythmic, coordinated activity in the brain and buccal ganglion of *Pleurobranchaea* arises from oscillatory circuits (or neurones) located only in the buccal ganglia' (Cohan & Mpitsos, 1983, p. 40).

The cause of these different interpretations cannot lie in the nature of the preparation used, since the isolated CNS preparation was used by Cohan & Mpitsos (1983) and in the present experiments. Instead we suggest two possible reasons for the primary observation of Cohan & Mpitsos that short-term interruption of ascending CBC activity blocked bursts of motor output in brain nerves. First, in their experiments the brain oscillation was activated *via* the buccal ganglion, by means of stomatogastric nerve stimulation. Therefore, when they blocked ascending activity they eliminated the only input to the brain that was activated using their methods. Under these conditions the cessation of brain oscillation is not unexpected. Second, Cohan & Mpitsos utilized motoneurone discharge as their primary index of brain oscillation. We have found here that the brain oscillator(s) is often uncoupled from the brain motoneurones that it drives (e.g. Fig. 5), and hence motoneurone discharge is alone inadequate as an index of central oscillatory activity.

Cohan & Mpitsos (1983) concluded further that the paracerebral command neurones cannot drive cyclic activity in the isolated brain (*ibid.* p. 35). The present experiments, however, illustrate that tonic intracellular stimulation of these identified feeding command interneurones invariably drives cyclic bursting in the command neurones (Fig. 5), and frequently elicits coupled rhythmic discharge in efferent nerves of the brain (Figs 3, 4). The negative result of Cohan & Mpitsos may have resulted from stimulating only one paracerebral command neurone (approximately 6% of the known cerebral command population) at a time. Cohan & Mpitsos (1983) also concluded that ascending CBC activity is necessary and sufficient to cause brain oscillation (*ibid.* p. 34). The present studies show that ascending activity is not necessary to cause brain oscillation, although the possibility that it is sufficient has not been tested. Finally Cohan & Mpitsos (1983) concluded that the corollary discharge neurones that ascend from the buccal ganglion supply the drive as well as the pattern for rhythmic brain activity (*ibid.* p. 35). The present experiments show that an independent neural oscillator(s) in the brain plays these roles, although a contribution from ascending activity has not been excluded. Published data do not permit rigorous assessment of the relative roles of brain and buccal oscillators, although previously we reported that the brain oscillator(s) is relatively labile in response to stomatogastric nerve stimulation in comparison with the buccal oscillator (Davis *et al.* 1973).

Coupling of independent neural oscillators

In combination with previous experiments, the present studies suggest two mechanisms by which coupling between brain and buccal oscillators of *Pleurobranchaea* might be accomplished. First, we have observed here that cutting the CBCs eliminates most of the previously demonstrated (Kovac *et al.* 1983) long-latency chemical polysynaptic excitation from the PSEs to the PCps. This finding indicates that most of this excitation is mediated by ascending neurone(s) from the buccal ganglion, i.e. the corollary discharge (CD) neurones. The cyclic bursts of

ascending action potentials that occur in the CD neurones (Davis *et al.* 1973) would by this mechanism deliver phasic entraining excitation to the command neurones of the brain. Second, we observed here that cyclic inhibition of the command neurones is weakened but not eliminated by isolating the brain (cf. Figs 2 and 3). Therefore, the 'cyclic inhibitory network' or CIN studied previously (Gillette *et al.* 1982) is at least partly located in the brain. Stimulation of single CD neurones causes polysynaptic inhibition of the brain command neurones (Gillette *et al.* 1978), perhaps *via* the CIN. Cyclic CD activity could by this arrangement deliver phasic entraining inhibition to the brain command neurones.

In addition to these postulated ascending excitatory and inhibitory coupling mechanisms, the command interneurones themselves discharge cyclic bursts of action potentials that descend to the buccal ganglion. This cyclic discharge also contains information on the timing of brain oscillator(s) activity, and could be used to couple the brain and buccal oscillations. Consistent with this hypothesis, intercalated bursts of impulses in the PCps advance or retard the phase of buccal oscillation (Gillette *et al.* 1982).

Maintenance of oscillatory capacity by ascending information

The present experiments show that the capacity of the command interneurones to elicit cyclic motor output declines rapidly after the CBCs are cut. Therefore ascending information from the buccal ganglion may help maintain the oscillatory capacity of the brain oscillator(s). Similarly, in the stomatogastric motor system of the lobster, input from the stomatogastric nerve contributes to the oscillatory capacity of neurones controlling both the gastric (Russell, 1976) and the pyloric (Russell & Hartline, 1978, 1982; Selverston & Miller, 1980) rhythms.

In the buccal motor system of *Pleurobranchaea*, the ascending information that is hypothesized to help maintain the oscillatory capacity of the brain is presumably carried by the CD neurones, but its nature is unknown. In the lobster stomatogastric system, dopamine has been circumstantially implicated as playing such a role (e.g. Anderson, 1977; Raper, 1979; Selverston & Miller, 1980; Kushner & Maynard, 1975; Friend, 1976). Similarly, various pharmacological agents sustain or release oscillatory neural activity in vertebrates (Ayers, Carpenter, Currie & Cinch, 1983; Buchanan & Cohen, 1982; Cohen & Wallen, 1980; Jankowska, Jukes, Lund & Lundberg, 1967*a,b*; Poon, 1980; see Grillner, 1981, for a review). Such a possibility requires further investigation in the buccal motor system of *Pleurobranchaea*.

Distributed neuronal oscillators controlling rhythmic behaviour

The present results show that rhythmic cerebro/buccal motor output in *Pleurobranchaea* is controlled by a separate and independent neural oscillator(s) located in the brain, in addition to a neural oscillator(s) demonstrated previously in the buccal ganglion (Davis *et al.* 1973). Cohan & Mpitsos (1983) have described a possible third source of neural oscillation, namely, the intrinsic oscillatory capacity of neurones in the CBCs, resulting presumably from properties of axonal membranes. The nerves of the two pedal ganglia also discharge cyclically and in phase with rhythmic motor output from the brain and buccal ganglion (Davis *et al.* 1973; Lee & Ligeois, 1974; G. Stoner & W. J. Davis, unpublished data). Therefore the pedal ganglia may contain yet additional neural oscillators for the control of this motor output. These studi

llectively indicate that the rhythmic cerebro/buccal motor programme(s) of *Pleurobranchaea* is generated by the coupling of multiple neuronal oscillators distributed widely throughout the nervous system.

The organization of this motor system therefore corresponds to the general principle that rhythmic patterns of behaviour are controlled by multiple coupled oscillators. This principle appears to apply to the leech swim system (Friesen, Poon & Stent, 1978; Kristan, 1974; Kristan & Calabrese, 1976; Kristan & Guthrie, 1977; Stent *et al.* 1978; Weeks, 1981), the leech heartbeat system (Peterson & Calabrese, 1982; Peterson, 1983*a,b*), the lobster stomatogastric ganglion (Miller & Selverston, 1982; Russell, 1976; Russell & Hartline, 1978, 1982; Selverston & Miller, 1980), the crustacean swimmeret system (Hughes & Wiersma, 1960; Wiersma & Ikeda, 1964; Stein, 1971, 1974), the swim system of *Tritonia* (e.g. Getting, 1983), and mammalian locomotor systems (Grillner, 1975, 1981). All of these motor systems share the common feature that coordinated, rhythmic movements are produced by independent neural oscillators distributed bilaterally and intersegmentally in the nervous system. Such decentralization of coupled neural oscillators presumably confers operational stability and control flexibility upon the corresponding motor systems, by enabling a degree of local central and reflex autonomy while nonetheless assuring coordination of widely separated body parts or appendages.

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REFERENCES

ANDERSON, W. W. (1977). Activation of a stomatogastric motor pattern generator by dopamine and L-DOPA. *Soc. Neurosci. Abstr.* **3**, 171.

AYERS, J. L., CARPENTER, G. A., CURRIE, S. & KINCH, J. (1983). Which behavior does the lamprey central motor program mediate? *Science, N.Y.* (in press).

BUCHANAN, J. T. & COHEN, A. H. (1982). Activities of identified interneurons, motoneurons and muscle fibers during fictive swimming in the lamprey and effects of reticulospinal and dorsal cell stimulation. *J. Neurophysiol.* **47**, 948–960.

COHAN, C. S. & MPITSOS, G. J. (1983). The generation of rhythmic activity in a distributed motor system. *J. exp. Biol.* **102**, 25–42.

COHEN, A. H. & WALLEN, P. (1980). The neuronal correlate of locomotion in fish. "Fictive swimming" induced in an *in vitro* preparation of the lamprey spinal cord. *Exp. Brain Res.* **41**, 11–18.

CROLL, R. P. & DAVIS, W. J. (1981). Motor program switching in *Pleurobranchaea*: I. Behavioural and electromyographic study of ingestion and ejection in intact specimens. *J. comp. Physiol.* **145**, 277–287.

CROLL, R. P. & DAVIS, W. J. (1982). Motor program switching in *Pleurobranchaea*: II. Ingestion and egestion in the reduced preparation. *J. comp. Physiol.* **147**, 143–154.

DAVIS, W. J. (1983). Neural consequences of experience in *Pleurobranchaea californica*. *J. Physiol, Paris* (in press).

DAVIS, W. J. & GILLETTE, R. (1978). Neural correlate of behavioral plasticity in command neurons of *Pleurobranchaea*. *Science, N.Y.* **199**, 801–804.

DAVIS, W. J., GILLETTE, R., KOVAC, M. P., CROLL, R. P. & MATERA, E. M. (1983). Organization of synaptic inputs to paracerebral feeding command interneurons of *Pleurobranchaea californica*. III. Modifications induced by experience. *J. Neurophysiol.* **49**, 1557–1572.

DAVIS, W. J., MPITSOS, G. J., SIEGLER, M. V. S., PINNEO, J. M. & DAVIS, K. B. (1974). Neuronal substrates of behavioral hierarchies and associative learning in the mollusk *Pleurobranchaea*. *Am. Zool.* **14**, 1037–1050.

DAVIS, W. J., SIEGLER, M. V. S. & MPITSOS, G. J. (1973). Distributed neuronal oscillators and efference co[illegible] in the feeding system of *Pleurobranchaea*. *J. Neurophysiol.* **36**, 258–274.

DELCOMYN, F. (1980). Neural basis of rhythmical behavior in animals. *Science, N.Y.* **210**, 492–498.

FRIEND, B. J. (1976). Morphology and location of dense-core vesicles in the stomatogastric ganglion of the lobster, *Panulirus interruptus*. *Cell Tissue Res.* **175**, 369–390.

FRIESEN, W. O., POON, M. & STENT, G. S. (1978). Neuronal control of swimming in the medicinal leech. IV. Identification of a network of oscillatory interneurons. *J. exp. Biol.* **75**, 25–43.

GETTING, P. A. (1983). Mechanisms of pattern generation underlying swimming in *Tritonia*. III. Intrinsic and synaptic mechanisms for delayed excitation. *J. Neurophysiol.* **49**, 1036–1050.

GILLETTE, R., KOVAC, M. P. & DAVIS, W. J. (1978). Command neurons in *Pleurobranchaea* receive synaptic feedback from the motor network they excite. *Science, N.Y.* **199**, 798–801.

GILLETTE, R., KOVAC, M. P. & DAVIS, W. J. (1982). Control of feeding motor output by paracerebral neurons in the brain of *Pleurobranchaea californica*. *J. Neurophysiol.* **47**, 885–908.

GRILLNER, S. (1975). Locomotion in vertebrates: central mechanisms and reflex interactions. *Physiol. Rev.* **55**, 247–304.

GRILLNER, S. (1981). Control of locomotion in bipeds, tetrapods and fish. In *Handbook of Physiology, The Nervous System II*, (ed. V. Brooks), pp. 1179–1236. Bethesda, Md.: American Physiological Society.

HUGHES, G. M. & WIERSMA, C. A. G. (1960). The coordination of swimmeret movements in the crayfish, *Procambarus clarkii* (Girard). *J. exp. Biol.* **37**, 657–670.

JANKOWSKA, E., JUKES, M. G. M., LUND, S. & LUNDBERG, A. (1967*a*). The effect of DOPA on the spinal cord. 5. Reciprocal organisation of pathways transmitting excitatory action to alpha motoneurons of flexors and extensors. *Acta physiol. scand.* **70**, 369–388.

JANKOWSKA, E., JUKES, M. G. M., LUND, S. & LUNDBERG, A. (1967*b*). The effect of DOPA on the spinal cord. 6. Half-centre organisation of interneurons transmitting effects from the flexor reflex afferents. *Acta physiol. scand.* **70**, 389–402.

KENNEDY, D. & DAVIS, W. J. (1977). The organization of invertebrate motor systems. In *Handbook of Physiology*, Section I, part 2, (eds J. M. Brookhart, V. B. Mountcastle, E. R. Kandel & S. R. Geiger), pp. 1023–1087. Bethesda, Md.: American Physiological Society.

KOVAC, M. P., DAVIS, W. J., MATERA, E. M. & CROLL, R. P. (1983). Organization of synaptic inputs to paracerebral feeding command interneurons of *Pleurobranchaea californica*. I. Excitatory inputs. *J. Neurophysiol.* **49**, 1517–1538.

KOVAC, M. P., DAVIS, W. J., MATERA, E. M. & GILLETTE, R. (1982). Functional and structural correlates of cell size in paracerebral neurons of *Pleurobranchaea californica*. *J. Neurophysiol.* **47**, 909–927.

KRISTAN, W. B., JR. (1974). Neural control of swimming in the leech. *Am. Zool.* **14**, 991–1001.

KRISTAN, W. B., JR. & CALABRESE, R. L. (1976). Rhythmic swimming activity in neurones of the isolated nerve cord of the leech. *J. exp. Biol.* **65**, 643–668.

KRISTAN, W. B., JR. & GUTHRIE, P. B. (1977). Acquisition of swimming behavior in chronically isolated single segments of the leech. *Brain Res.* **131**, 191–195.

KUSHNER, P. D. & MAYNARD, E. (1975). Monamine histochemistry of the crustacean stomatogastric nervous system. *Soc. Neurosci. Abstr.* **1**, 577.

LEE, R. M. & LIGEOIS, R. J. (1974). Motor and sensory mechanisms of feeding in *Pleurobranchaea*. *J. Neurobiol.* **5**, 545–565.

MCCLELLAN, A. D. (1982*a*). Movements and motor patterns of the buccal mass of *Pleurobranchaea* during feeding, regurgitation and rejection. *J. exp. Biol.* **98**, 195–211.

MCCLELLAN, A. D. (1982*b*). Re-examination of presumed feeding motor activity in the isolated nervous system of *Pleurobranchaea*. *J. exp. Biol.* **98**, 213–228.

MILLER, J. P. & SELVERSTON, A. I. (1982). Mechanisms underlying pattern generation in lobster stomatogastric ganglion as determined by selective inactivation of identified neurons. II. Oscillatory properties of pyloric neurons. *J. Neurophysiol.* **48**, 1378–1391.

PETERSON, E. L. (1983*a*). Generation and coordination of heartbeat timing in the medicinal leech. I. Oscillation in isolated ganglia. *J. Neurophysiol.* **49**, 611–626.

PETERSON, E. L. (1983*b*). Generation and coordination of heartbeat timing in the medicinal leech. II. Intersegmental coordination. *J. Neurophysiol.* **49**, 627–638.

PETERSON, E. L. & CALABRESE, R. L. (1982). Dynamic analysis of a rhythmic neural circuit in the leech *Hirudo medicinalis*. *J. Neurophysiol.* **47**, 256–271.

POON, M. L. T. (1980). Induction of swimming in lamprey by L-DOPA and amino acids. *J. comp. Physiol.* **136**, 337–344.

RAPER, J. (1979). Non-impulse mediated synaptic transmission during the generation of a cyclic motor program. *Science, N.Y.* **205**, 304–306.

RUSSELL, D. F. (1976). Rhythmic excitatory inputs to the lobster stomatogastric ganglion. *Brain Res.* **101**, 582–588.

RUSSELL, D. F. & HARTLINE, D. K. (1978). Bursting neural networks: a reexamination. *Science, N.Y.* **200**, 453–456.

[illegible]SELL, D. F. & HARTLINE, D. K. (1982). Slow active potentials and bursting motor patterns in pyloric network of the lobster, *Panulirus interruptus*. *J. Neurophysiol.* **48**, 914–937.

SELVERSTON, A. I. & MILLER, J. P. (1980). Mechanisms underlying pattern generation in lobster stomatogastric ganglion as determined by selective inactivation of identified neurons. I. Pyloric system. *J. Neurophysiol.* **44**, 1102–1121.

STEIN, P. S. G. (1971). Intersegmental coordination of swimmeret motoneuron activity in crayfish. *J. Neurophysiol.* **34**, 310–318.

STEIN, P. S. G. (1974). Neural control of interappendage phase during locomotion. *Am. Zool.* **14**, 1003–1016.

STENT, G. S., KRISTAN, W. B., JR., FRIESEN, W. O., ORT, C., POON, M. & CALABRESE, R. L. (1978). Neuronal generation of the leech swimming movement. *Science, N.Y.* **200**, 1348–1356.

WEEKS, J. C. (1981). The neuronal basis of leech swimming: Separation of swim initiation, pattern generation, and intersegmental coordination by selective lesions. *J. Neurophysiol.* **45**, 698–723.

WIERSMA, C. A. G. & IKEDA, K. (1964). Interneurons commanding swimmeret movements in the crayfish, *Procambarus clarkii* (Girard). *Comp. Biochem. Physiol.* **12**, 509–525.