J. exp. Biol. **102**, 25–42, 1983
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THE GENERATION OF RHYTHMIC ACTIVITY IN A DISTRIBUTED MOTOR SYSTEM

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(*Received 18 March 1982—Accepted 29 July 1982*)

SUMMARY

Rhythmic activity that is distributed to the brain and buccal ganglia and which underlies several types of behaviour, can be evoked from isolated nervous systems of *Pleurobranchaea californica* by tonic nerve stimulation. The experiments presented here were designed to test whether this rhythmic activity is produced by independent neuronal oscillators located in each ganglion or whether the rhythmic activity arises from a single oscillatory locus in the buccal ganglion and is transmitted passively to the brain. By interrupting the conduction of activity in the cerebrobuccal connectives (CBC) between brain and buccal ganglia we show that motor output from the brain depends on sustained, cycle to cycle input from the buccal ganglion and cannot be reset with respect to the buccal activity. The production of rhythmic activity in the brain depends on the generation of rhythmic activity in the buccal ganglia whether the rhythms are activated by stimulation of buccal roots or paracerebral command cells in the brain. Simultaneous intracellular recordings from brain motoneurones and buccal interneurones which project to the brain indicate that these interneurones provide both the drive and the pattern for rhythmic motor output in the brain. Tonic stimulation of the CBC can produce rhythmic activity in isolated brains in which all nerve roots and connectives have been cut. This can be explained by the fact that tonic stimulation of the connectives is transformed into phasic activity by the axons within the connective. We conclude therefore, that rhythmic, coordinated activity in the brain and buccal ganglia of *Pleurobranchaea* arises from oscillatory circuits that are located only in the buccal ganglia.

INTRODUCTION

The present understanding of the generation of behaviour by nervous systems has arisen primarily from studies on relatively stereotyped movements such as swimmeret beating (Hughes & Wiersma, 1960; Davis, 1969*a*,*b*,*c*), postural control (Evoy & Kennedy, 1967; Kennedy, Evoy, Dane & Hanawalt, 1967; Bowerman & Larimer, 1974*a*,*b*), walking (Pearson, 1972), and escape responses (Wine, 1977*a*,*b*; Zucker, Kennedy & Selverston, 1971) in arthropods, swimming in annelids (Kristan, 1974;

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Key words: Neuronal oscillators, molluscan feeding, coordinated motor output.

Stent *et al.* 1978), and swimming (Willows, Dorset & Hoyle, 1973*a*,*b*; Getting, 1977) and feeding in molluscs (Kater, 1974; Davis, Siegler & Mpitsos, 1973; Kupfermann & Cohen, 1971; Gelperin, Chang & Reingold, 1978). Traditionally, individual sensory-to-motor neurocircuits have been isolated for study in experiments in which a specific stimulus is applied to produce a definable response. Some functions of neurones, such as command (Kupfermann & Weiss, 1978), trigger (Getting, 1975), coordination (Stein, 1971, 1974), and oscillation functions (Wilson, 1961; Delcomyn, 1980) have emerged from such studies and extended the notion of the neurocircuit beyond that of the simple reflex. Nonetheless, this experimental approach has forced a reductionist perspective that, although heuristically useful, views behaviour as a repertoire of independent responses and is inconsistent with the observed behaviour of animals.

The behaviour of freely moving animals appears as smooth transitions between different responses rather than sequences of independent movements; one behavioural pattern 'blends' into another such that the execution of one response is affected by the attempt to produce another (Bellman, 1979). Animals continuously receive many different stimuli simultaneously, some of which may require contradictory responses, and all of which produce some effect in the nervous system. An understanding of the integrative properties of the nervous system, and even of the applicability of the extant concepts of neurone functions and circuits to the analysis of behaviour, requires a holistic perspective in which the interactive effects produced by many stimuli and different motor activities are considered.

We have studied the neuronal basis of different types of behaviour that involve relatively similar movements in the marine gastropod, *Pleurobranchaea*. The buccal and anterior head structures of *Pleurobranchaea*, namely, the buccal mass, radula, mouth, lips, jaws, and oral veil, are used in at least five types of behaviour: feeding, regurgitation (active and passive phase), rejection, defensive bite, and self and interanimal gill grooming. At least three of these – feeding, regurgitation, and rejection – can be reliably elicited and have been studied extensively (McClellan, 1982*a*). All five types of behaviour involve similar observed movements of the buccal structures, yet the consequences of these movements are quite different. For example, during feeding, a substance is sequentially ingested and swallowed by cyclical movements, and during rejection the ingested substance is sequentially expelled by movements that are outwardly similar. Although some muscles must be activated differently in the two sets of movements to account for the different effects, there is much similarity in the underlying motor programmes recorded from muscles in behaving animals and from nerves in isolated nervous systems. In fact, at present it is possible to distinguish only two rhythmic patterns, one attributable to the active phase of regurgitation and another which may represent aspects of the different types of behaviour (McClellan, 1982*a*,*b*).

The structures which participate in the above types of behaviour are innervated primarily by the buccal and cerebropleural (brain) ganglia. The buccal ganglia innervate the intrinsic muscles of the buccal mass which are responsible for movements of the radula and jaws, whereas the brain innervates the extrinsic buccal muscles involved in lip, mouth, oral veil, and proboscis movements. These data are based on experiments in which (1) the distal ends of cut nerves were stimulated extracellularly

while activity in the buccal muscles was observed, and (2) muscle potentials were found to occur at constant latency in relation to identified action potentials which were recorded from the innervating nerves (Davis *et al.* 1973; Lee & Liegeois, 1974). Since the different behavioural sequences require movements of intrinsic and extrinsic muscles, there must be coordination of activity between the brain and buccal ganglia so that the appropriate motor patterns are produced. Moreover, since these patterns are expressed in the motor output of deafferented brain and buccal ganglia (McClellan, 1982*b*) it is possible to use isolated preparations to enquire into the principal mechanisms of coordination.

Previous studies have suggested that patterns of rhythmic activity in the brain and buccal ganglia require coordination of independent neuronal oscillators located in each ganglion (Davis *et al.* 1973, 1975). In the present paper, however, it will be shown that oscillatory activity is generated only in the buccal ganglion, and that this provides the cyclic drive for responding centres in the brain which are not inherently oscillatory. In the following study (Cohan & Mpitsos, 1983) it will be shown that the different motor patterns which can be obtained in isolated nervous systems are produced by the selective action of an intermediary group of functionally heterogeneous neurones in the buccal ganglion, buccal-cerebral interneurones (BCI), that connect the driving with the responding loci. A third paper (C. S. Cohan & G. J. Mpitsos, in preparation), will discuss how the multifunctional properties of neurones in the nervous system of *Pleurobranchaea* allow the drive of an oscillator to be expressed in a variety of motor patterns and will also suggest mechanisms by which different motor patterns and smooth transitions between them might be produced by a limited number of neurones.

MATERIALS AND METHODS

Specimens of *Pleurobranchaea californica* were obtained from Pacific Biomarine Labs (Venice, California). These animals ranged in size from 100–300 ml body volume and were housed individually in a circulating seawater system. Preparations of the nervous system, consisting of buccal, cerebral, pedal, visceral and stomatogastric ganglia (Fig. 1) were dissected from the animals and pinned through their connective tissue sheaths to a Sylgard (Corning) platform in a Lucite preparation chamber. After desheathing the nerves, suction electrodes were attached for recording and stimulating. Ganglia were bathed in artificial sea water (Instant Ocean) supplemented with 1% dextrose (denoted by ASW) and maintained at 11 °C by a circulating cooler (Haake).

Conduction blocks

Sucrose perfusion

Neuronal activity in the cerebrobuccal connective (CBC) was reversibly blocked by two different methods. For long-term blocks (greater than 1 min) the CBC were sucked into a small perforation made in a polyethylene tube. When a suitable length of nerve was within the tube, the perforation was sealed with Vaseline and overlaid

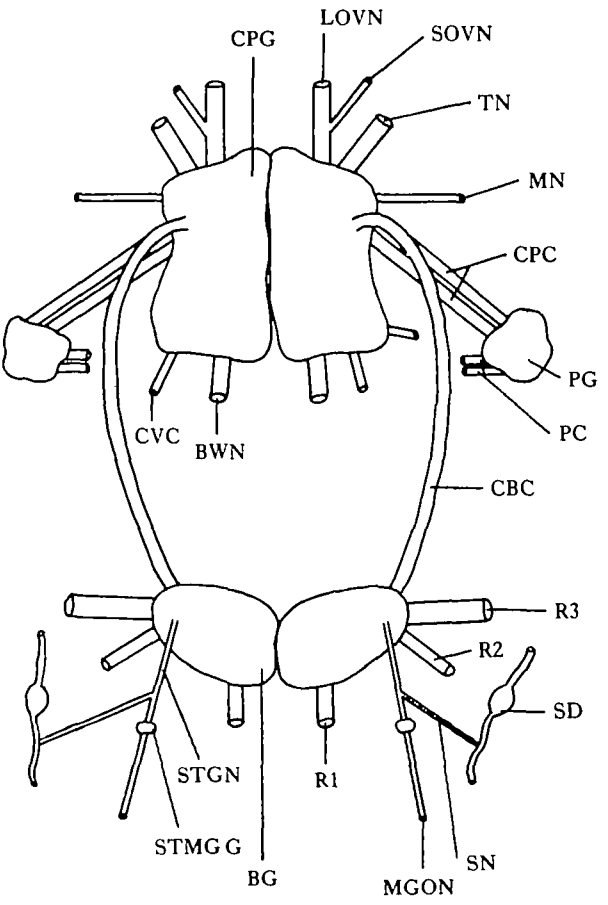

Fig. 1. Drawing of the ventral surface of an isolated nervous system. Abbreviations are as follows: LOVN, large oral veil nerve; SOVN, small oral veil nerve; TN, tentacle nerve; MN, mouth nerve; CPC, cerebropedal connective; CVC, cerebrovisceral connective; BWN, body wall nerve; PC, pedal connective; CBC, cerebrobuccal connective; R3, R2 and R1 are the third, second and first roots of the buccal ganglion, respectively; STGN, stomatogastric nerve; SN, salivary nerve; MGON, medial gastroesophageal nerve; STMG G, stomatogastric ganglion; BG, buccal ganglion; CPG, cerebropleural ganglion; PG, pedal ganglion; SD, salivary duct.

with a small glass coverslip so as to form a sandwich containing portions of the intact nerves surrounded by the jelly. This provided the structural rigidity necessary for maintaining the leak-proof inner chamber of the polyethylene tube, especially when solutions were changed. A syringe was used to apply negative pressures for securing the nerves to the perforation and for drawing experimental and control media through the tube to bathe the small loops of the CBC. When the bathing sea water solution within the tube was replaced by a 1 M-sucrose solution to which a small amount of fast green FCF dye was added as a marker, complete and reversible block of CBC activity was achieved within 2–3 min. Neuronal activity reappeared within 5 s after the sucrose solution had been replaced by ASW.

Anodal block

Anodal blocks were used when immediate conduction block of CBC activity was required. This was achieved by applying anodal current to the nerve via a suction electrode, exactly as for an *en passant* recording. A nearby indifferent lead served as the positive current source. Its placement did not affect block efficacy. Activity within the nerve was monitored by an appropriately placed additional electrode either upstream or downstream from the blocking electrode. The blocking electrode could be switched easily to record mode if so desired.

Square wave, d.c., constant current pulses of approximately 10 μA in amplitude and of durations equal to the intended blocking time were used. Generally, the amplitude of the blocking current was set at a value just greater than threshold for conduction block. This point could easily be found by slowly increasing the amplitude of the blocking current while applying short (0·5 s) d.c. pulses. This caused stimulation of the nerve, at first, until the current at the anode became large enough to suppress conduction. This method resulted in the least amount of anode break excitation when the current was switched off. Greater amplitude currents progressively increased the amplitude and duration of break excitation and irreversibly damaged the nerve. With anodal block, activity in the CBC could be blocked immediately at stimulus onset and was reversible for durations up to 1 min.

Identification of motor patterns

Rhythmic motor patterns can be obtained reliably from the isolated nervous system (Fig. 1) by a variety of methods, among which are tonic stimulation of the stomatogastric (Davis *et al.* 1973) and gastro-oesophageal (McClellan, 1982*b*) nerves, and by tonic depolarization of the paracerebral cells in the brain (Gillette, Kovac & Davis, 1978). Originally, all such patterns were presumed to represent the feeding behaviour. More recent work (McClellan, 1980, 1982*a*), however, has suggested that the buccal structures which are innervated by these ganglia are used in at least five types of behaviour. In accordance with this it has been shown that stimulation of the medial gastro-oesophageal (MGON) or stomatogastric (STGN) nerves at low current levels evokes motor patterns that may represent rejection and the passive phase of regurgitation as well as feeding, whereas higher current levels evoke a second type of motor pattern which has been identified with the active phase of regurgitation (McClellan, 1982*b*). We shall therefore refer to the first type of motor output as the primary pattern rather than as the feeding motor pattern, and to the second type of motor output as the vomiting motor pattern.

Identification of cells

Cells were identified as having processes in a particular nerve if the following criteria were met: (1) the general location of cell bodies studied intracellularly during an experiment coincided with cell bodies localized by cobalt backfills of the nerve; (2) depolarization of the cell soma produced orthodromic action potentials with constant latency in the nerve for spike frequencies greater than 10 Hz; and (3) stimulation of the nerve root which contained the axon of the cell produced an antidromic action

potential in the cell body of the neurone. These antidromic potentials followed on for one at frequencies of 10 Hz.

Intracellular recording

Ganglia were prepared for intracellular recording by removing their connective tissue sheath in a high osmolarity (1500 mosM) sucrose-supplemented bathing solution to preserve ganglionic morphology. Before recording, the bathing solution was replaced several times with ASW. Single and double barrelled glass capillary micropipettes were filled with 3 M-KCl and beveled (Ogden, Citron & Pierantoni, 1978) to a final impedance of 5–20 MΩ. Activity was displayed via capacitively compensated amplifiers (McClellan, 1980) on an oscilloscope and simultaneously stored on magnetic tape using an FM tape recorder (Vetter). Records were photographed with a 35 mm oscilloscope camera and image intensifier (McClellan, 1980). A PDP-11 minicomputer was used to analyse data. Graphs were constructed using Fortran programmes (Cohan, 1980) and a Hewlett-Packard plotter.

RESULTS

The primary and vomiting motor patterns (defined in Materials and Methods) both exhibit cyclical motor activity in the brain that is phase-locked with alternating bursts of activity in roots R1 and R3 of the buccal ganglion (Fig. 2). The only brain roots that consistently fire cyclically are the SOVN and MN. Both appear to be primarily motor nerves, as shown by recordings that were made from the distal nerve stumps during tactile and chemosensory stimulation of the oral veil (Lee & Liegeois, 1974). Cobalt backfills of the proximal nerve stumps indicate that these nerves contain about 20–30 neurones. In the primary pattern, bursts of activity in the SOVN and MN characteristically occur in phase with bursts in R3 (Figs 2A, 3), whereas in the

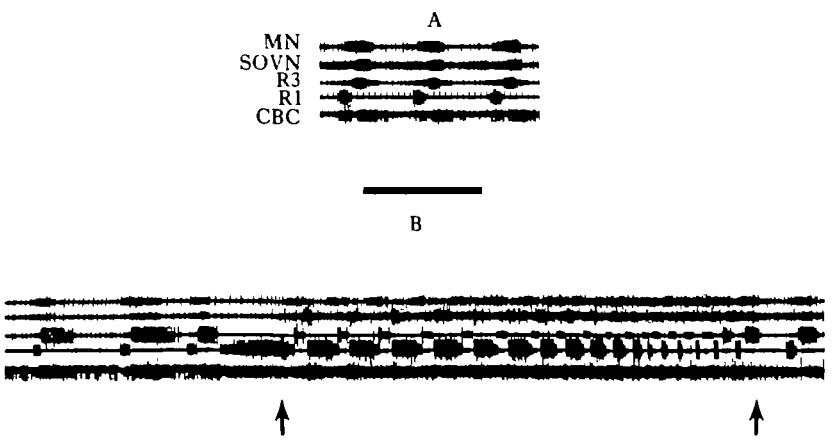

Fig. 2. Two different motor patterns elicited from isolated nervous systems by tonic MGON stimulation. (A) Primary pattern and (B) vomiting motor pattern (between arrows) which interrupts the primary pattern. During the vomiting pattern notice increase in frequency of buccal rhythm (R3 and R1), tonic activity in brain roots and bursts in SOVN and CBC which occur in-phase with R1 activity in (B). Calibration: 20 s.

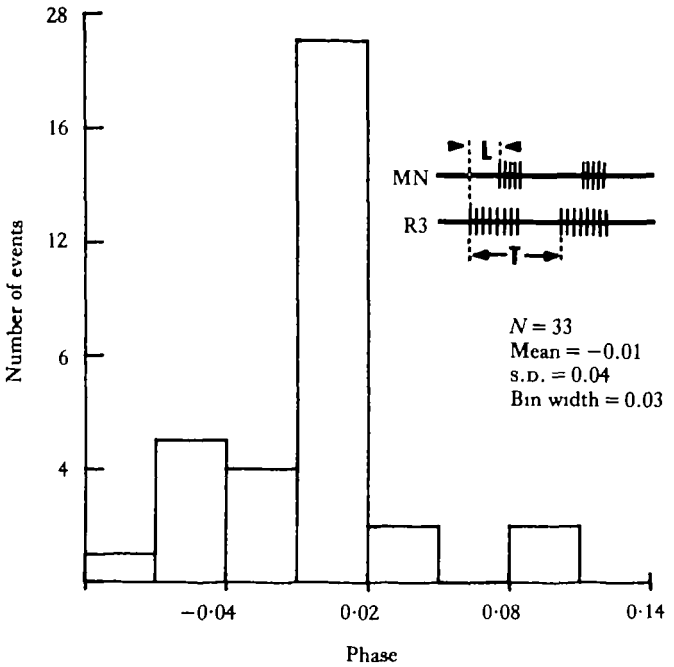

Fig. 3. Histogram of phase position of MN bursts relative to R3 bursts during MGON-evoked primary rhythms. Phase is defined as latency (L) of the onset of the burst in MN divided by the cycle time (T). Negative phases indicate occurrence of MN burst prior to onset of R3 activity. Data from a single representative animal.

vomiting pattern, the MN becomes more tonically active and the SOVN responses occur in phase with R1 rather than R3 (Fig. 2B).

In order to understand the integrative mechanisms that produce these coordinated patterns, and eventually to understand the mechanisms that underlie the types of behaviour that are related to these motor patterns, it is necessary to determine whether the cyclical characteristics of the brain and buccal ganglion motor outputs occur as the result of phasing between independent oscillators or whether the activity of one ganglion arises passively in response to cyclical input from the other. In the following sections we shall enquire into the ability of each ganglion to produce cyclical activity independently of the other by applying sucrose gap and anodal current blocks to the CBC, which are the only connections between the brain and buccal ganglion in the isolated preparation. We shall also examine some of the properties of the interneurones that interconnect the two ganglia, and enquire into previous evidence which originally suggested that there may be separate oscillators.

Interruption of activity in the CBC during ongoing motor patterns

Anodal blocks, applied to the CBC during rhythms that were evoked by MGON stimulation, stopped brain motor output but allowed the buccal rhythm to continue (Fig. 4A). The same effect was always produced, even when the blocks were applied for durations of a minute or more, which exceeds the cycle period by 5–6 times, and whether they were applied during the primary or vomiting motor pattern (Fig. 4B). Sustained brain motor output, therefore, is dependent on continued drive from the

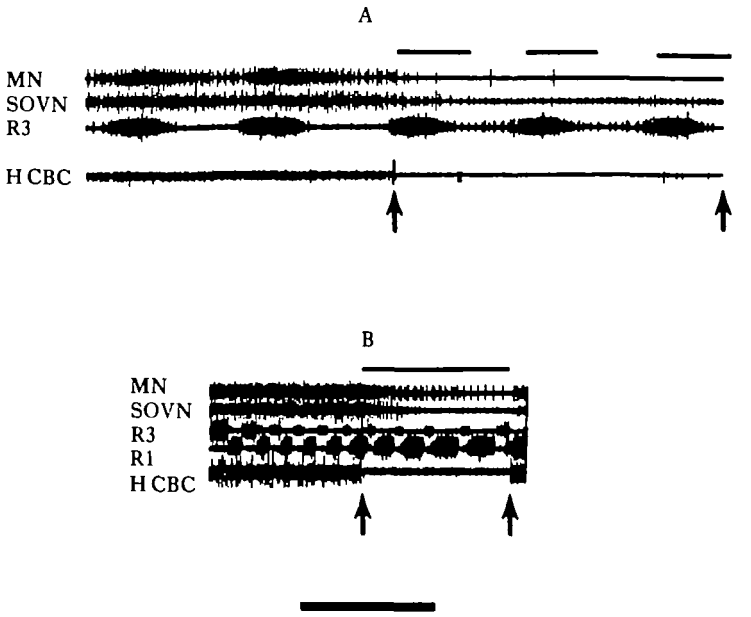

Fig. 4. Effect of long duration anode block of CBC activity on bursts of activity in roots of the brain. Activity in brain roots (top two traces) ceases when CBC's are blocked (between arrows) either during primary pattern (A) or during a bout of vomiting (B). Rhythms were evoked by tonic MGON stimulation. Here and in subsequent figures H CBC activity is recorded at a point between the site of block and the brain in order to monitor block effectiveness. Bars above records indicate where brain activity would have occurred. Calibration: (A) 10 s, (B) 20 s $N = 19$ in 11 animals for (A) and $N = 7$ in 5 animals for (B).

buccal ganglion when rhythms are activated by stimulation of nerves of the buccal ganglion, namely the MGON. The remaining experiments were performed solely on the primary pattern because its longer time course permitted more extensive experimental analysis.

In further contrast to what would be expected of phase-locked oscillators, short duration blocks that were applied during different portions of the buccal cycle did not reset or shift the phase of the brain output in the subsequent cycles (Fig. 5). However, blocks that were applied during the entire R3 phase deleted one set of bursts in the output of the brain (Fig. 6A). If the onset of the blocks occurred after the R3 burst had begun, the brain response abruptly ceased (Fig. 6B), and when the block was removed before the end of the R3 burst, the brain activity immediately commenced (Fig. 6C). This indicates that continued drive from the buccal ganglion is necessary for even a single, complete burst in the brain roots and that the driving input to the brain occurs during the R3 phase of buccal activity.

The relative importance of the temporal location of CBC activity to the generation of bursts in the brain was determined by experiments in which CBC blocks were delivered sequentially in consecutive cycles of buccal activity. Blocks of the CBC during the entire R3 phase in consecutive cycles suppressed all brain output (Fig. 7A), but, conversely, blocks during the entire phase between the R3 bursts in consecutive cycles appeared to have no effect on the brain output (Fig. 7B). Thus, CBC activity which occurs during the R3 phase of the rhythm is both necessary and sufficient for

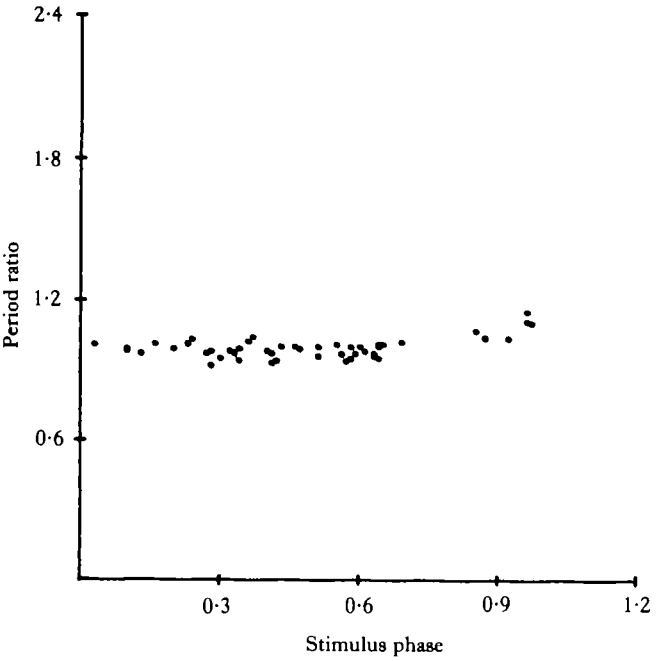

Fig. 5. Phase response curve. Plot of the effect of anodal blocks on the onset of MN bursts when the blocking pulse is applied at different phase positions of the rhythm cycle. The stimulus phase is defined as the latency of the block onset, measured from the beginning of the previous MN burst, divided by the R3 period. The period ratio is the ratio of the perturbed MN period to the corresponding R3 period. ($N = 50$, 4 animals.)

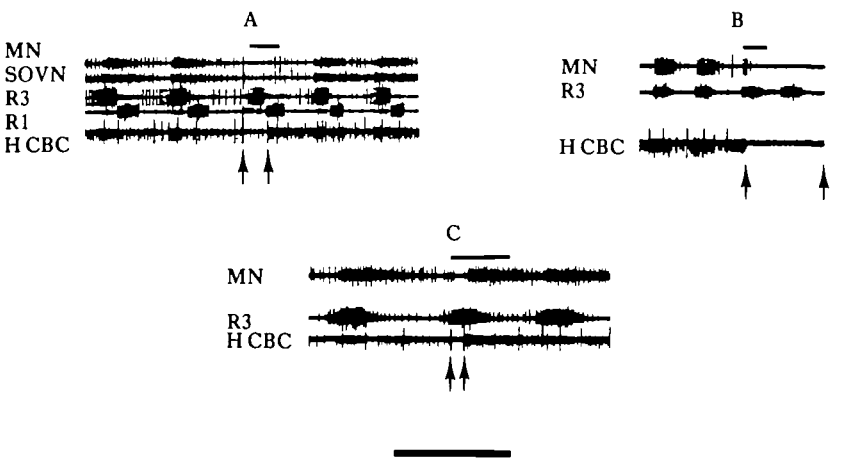

Fig. 6. Driving input to the brain in primary pattern occurs during the R3 portion of the cycle (A). When a CBC blocking pulse (between arrows) is applied for the full duration of the R3 burst, the brain root burst which would have occurred (bar) is absent. Note also that the cycle following the block is unaffected ($N = 24$, 6 animals). A blocking pulse (between arrows) which occurs after (B) or before (C) the R3 burst has begun deletes portions of the brain root burst. Bars above figures indicate mean position and duration of unperturbed brain burst. Calibration: (A), (B) 20 s; (C) 10 s.

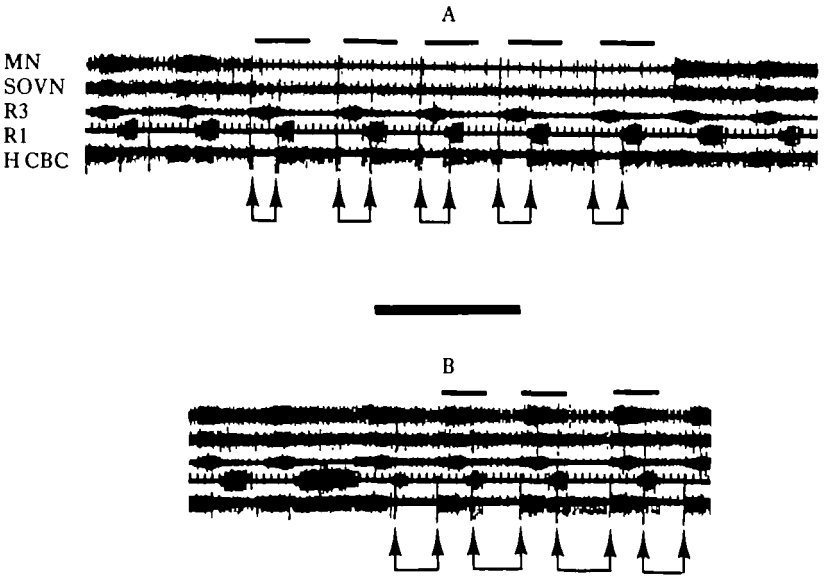

Fig. 7. Consecutive CBC blocks (between arrows) applied during an MGON-evoked primary rhythm demonstrate that CBC activity during the R3 phase of the buccal cycle is (A) necessary and (B) sufficient for the brain rhythm. Same preparation in (A) and (B). Bars as in Fig. 6. ($N = 5$ animals.) Calibration: 20 s.

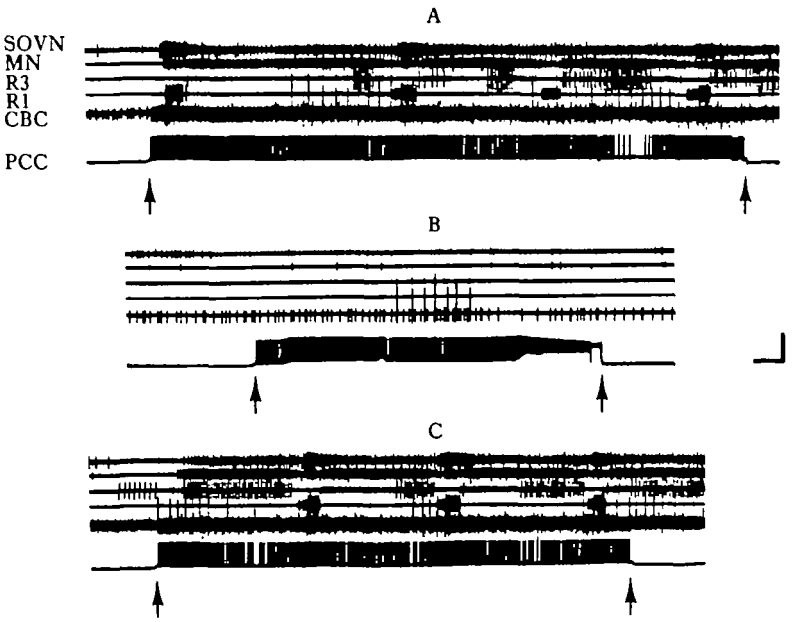

Fig. 8. PCC stimulation (A) before, (B) during, and (C) after activity in the CBC is blocked bilaterally with a sucrose gap. Arrows denote period of intracellular depolarization. CBC activity which was recorded on the buccal ganglion side of the block. The same results were also obtained for contralateral brain root activity (not shown) ($N = 10$, 6 animals.) Calibration: 150 mV, 5 s.

brain root bursts. These findings show, furthermore, that the drive as well as the timing for brain output occurs during the R3 phase of the buccal cycle, and that the production of rhythmic brain output requires rhythmic input from the buccal ganglion.

In the experiments described above, the motor patterns were elicited by electrical stimulation of buccal nerves. Although cyclical responses are difficult to produce by stimulation of afferent roots of the brain, they can be elicited by stimulation of neurones that are located in the brain and that send their axons to the buccal ganglion via the CBC (Davis *et al.* 1973; Gillette *et al.* 1978). The most effective of these neurones are the paracerebral cells (PCC), and the coordinated rhythm that is produced by tonic depolarization of one of the PCC is shown in Fig. 8A. When conduction in the CBC is blocked, depolarization of the PCC does not evoke activity in either the brain or the buccal ganglion (Fig. 8B). The return of conduction in the CBC by perfusion with ASW restores activity in both ganglia (Fig. 8C). Findings such as these show that the PCC cannot drive motor output in the brain by local connections they might have there. They also show that, regardless of whether the rhythms are activated by stimulation of the brain or buccal ganglion, the production of cyclical activity in the brain depends on the generation of cyclical activity in the buccal ganglion.

Buccal interneurones provide the drive for brain motoneurones

The general class of neurones which we shall refer to as the buccal-cerebral interneurones (BCI; see Discussion) provide the only connection from the buccal ganglion to the brain in the isolated nervous system (Davis *et al.* 1973). Simultaneous intracellular recordings from cells in both ganglia showed that, although there are differences among the BCI in the strength of their effects on the brain motoneurones, some are closely coupled as indicated by one-to-one postsynaptic potentials in the motoneurones with respect to action potentials in the BCI (Fig. 9A). Such pairs of cells were found in three of nine different preparations in which simultaneous penetrations were made to find these cells. Recordings that were obtained during rhythms showed that hyperpolarization of one such BCI suppressed the phasic activity of some motoneurones while others in the preparation continued to fire (Fig. 9B). These data indicate that the BCI supply the drive as well as the pattern for rhythmic activity in the brain motoneurones and also that several BCI are coactivated to produce the motor output. The close association between the BCI and motoneurones suggests, in support of the findings presented in the previous section, that a brain oscillator is not interposed between them.

From the findings presented here and in the preceding section, we conclude that the rhythmic activity which is generated in the buccal ganglion, and conveyed to the brain by the BCI, is both necessary and sufficient for producing the cyclic motor output of the brain. That is, rhythmic patterns of coordinated motor output of the brain and buccal ganglion do not appear to be produced by the phasing of independent oscillators, but rather that the output of the brain occurs in response to rhythmic input from the buccal ganglion.

The relationship of the BCI to the central pattern generator (CPG) in the buccal ganglion is presently unclear. Previous investigations showed that BCI activity that

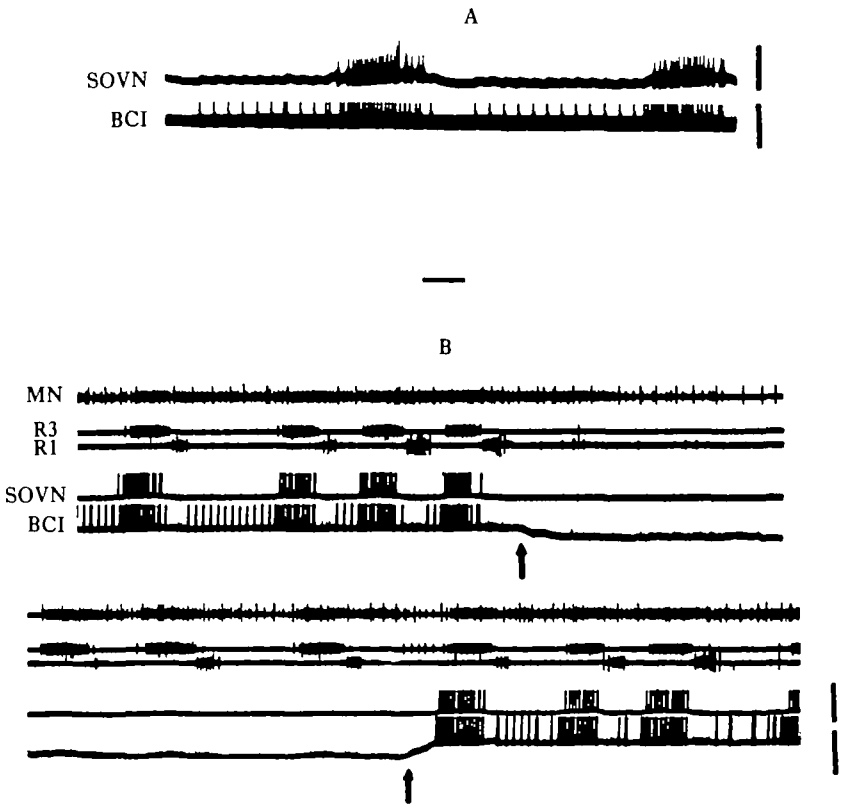

Fig. 9. BCI drive brain motoneurones that are active during the primary pattern. (A) Postsynaptic potentials in an SOVN motoneurone follow 1-1 spikes in a BCI. (B) Hyperpolarization (between arrows) of this BCI during an MGON-evoked primary rhythm is correlated with absence of activity in the SOVN motoneurone. Other brain motoneurones continue to fire phasically. Absence of buccal and brain activity which occurs just after hyperpolarization begins probably is not causally related and is within the normal variation of the rhythm period. Continuous record. Same preparation in (A) and (B). ($N = 3$, 3 animals.) Calibration: (A) upper 30 mV, lower 150 mV, 3 s; (B) upper 180 mV, lower 120 mV, 6 s.

was recorded in the CBC can occur independently of rhythmic activity in the buccal roots (Davis *et al.* 1973). In addition, we have found that intracellular stimulation of BCI never evoked rhythmic activity from buccal roots. Thus, the BCI may not be part of the CPG or else, insufficient numbers of BCI were stimulated to show this effect. Nonetheless, stimulation of some BCI did produce tonic activity in the motor roots of the buccal ganglion (Cohan & Mpitsos, 1983).

Rhythmic activity in isolated brain ganglia

The original suggestion that the brain of *Pleurobranchaea* contains an oscillator came from the observation that tonic stimulation of the proximal stump of the CBC produced rhythmic motor output from brains in which all nerve roots had been severed (Davis *et al.* 1973). This type of test is used traditionally to determine the presence of a central oscillator in deafferented nervous systems, and, on repeating the

riginal experiments, we obtained the same results (Fig. 10). We found that the production of rhythmic brain output depends critically on the parameters of the electrical stimuli that are applied to the CBC. By positioning a suction recording electrode between the site of stimulation on the CBC and the brain, it was observed that cyclic brain output occurred when the stimulus parameters were near the threshold for evoking action potentials in the CBC. Moreover, we observed that bursts in the CBC preceded or occurred simultaneously with the bursts in the brain (Fig. 11). The shorter latency of the bursts in the CBC suggested the possibility that the axons of the CBC themselves are capable of generating rhythmic bursts of activity when they are tonically stimulated. Surprisingly, this proved to be the case, as shown by experiments in which isolated segments of the CBC were stimulated at one of their cut ends and the resulting activity that was evoked was recorded at the opposite end. The cycle period of bursts in the isolated segments of the CBC (Fig. 12) was similar to that of the brain output and the stimulus parameters that produced the bursts in the CBC were the same as those which produced the bursts in isolated brains. We propose, therefore, that the cyclical output of isolated brains is driven by the cyclical

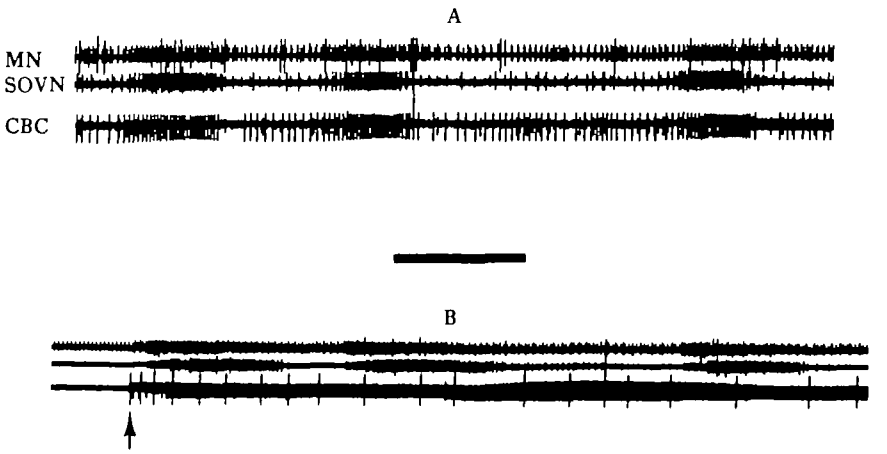

Fig. 10. Rhythmic activity produced by isolated brains after cutting all connectives to other ganglia. (A) Spontaneous rhythm and (B) rhythm evoked by tonic stimulation (at arrow) of the ipsilateral CBC with 1 ms, 10 μA pulses at 10 Hz. Calibration: 20 s.

Fig. 11. During tonic CBC stimulation (10 Hz) in isolated brains (all roots cut), bursts in the CBC overlap with bursts in brain roots. Continuous record. Stimulation was begun before record. Calibration: 20 s.

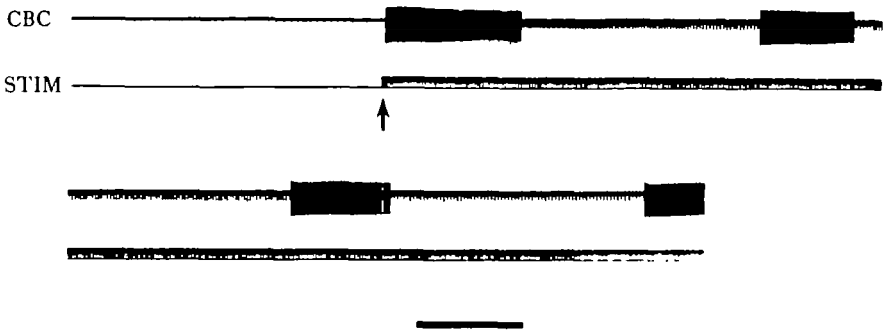

Fig. 12. Tonic stimulation (10 Hz) at one end of an isolated segment of CBC, severed from brain and buccal ganglia, results in phasic activity at the other end. ($N = 6$ animals.) Calibration: 20 s.

activity in the CBC. Thus, the original conclusion may well have been based on findings that were produced by an artifact of stimulation rather than by a centrally programmed oscillator in the brain.

One of the implications of the above proposal is that the motor units which are activated by CBC stimulation in isolated brain ganglia should be different from those in rhythms produced by other methods of stimulation. This is because electrical stimulation of the CBC would indiscriminately activate not only the BCI but also other axons in the CBC, whereas electrical stimulation of the MGON, for example, would activate the BCI as required by the buccal rhythm. Accordingly, not only are there considerable differences between the units that comprise the CBC-evoked and MGON-evoked rhythms, but these units are also different from those of spontaneously occurring rhythms (Fig. 13). Intracellular records from brain motoneurones show, moreover, that individual units can have different response properties, depending on the type of pattern that occurs and how it is activated (Fig. 14). Thus, while the patterns of bursts that are elicited by different methods of stimulation may often appear similar, the bursts themselves can have different microstructures since a variety of units may selectively come into play or respond differently.

DISCUSSION

Previous investigations in which rhythmic activity was evoked from isolated ganglia led to the conclusion that rhythmic motor patterns in *Pleurobranchaea* are produced by independent neuronal oscillators that are distributed in the brain and buccal ganglia (Davis *et al.* 1973). However, our attempts to uncover the mechanisms by which the putative oscillators are coordinated have revealed additional data which indicate that only a single oscillator drives the distributed motor activity. We have shown here that: (1) the production of cyclical brain output, and even the production of one complete burst, requires continual input to the brain from the buccal ganglion; (2) the phase of the brain output cannot be reset with respect to the phase of the buccal output; (3) rhythmic brain output requires rhythmic buccal input whether activity is evoked by stimulation of brain or buccal ganglia; (4) the BCI, which carry the buccal information to the brain, appear to provide both the drive and the pattern of activity to the brain motoneurones. In contrast to coupled oscillators, it has also been shown

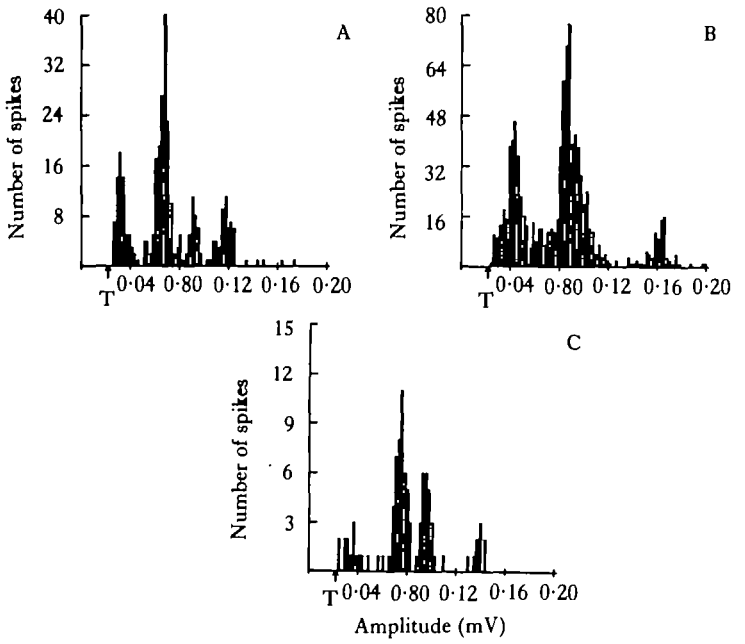

Fig. 13. Spike height histograms of MN motoneurones that became active during MN bursts which occurred (A) during an MGON-evoked primary pattern in an intact buccal-cerebral preparation or (B) spontaneously and (C) as a result of tonic CBC stimulation in isolated brains. All histograms are from the same preparation. T represents threshold value of spike amplitudes.

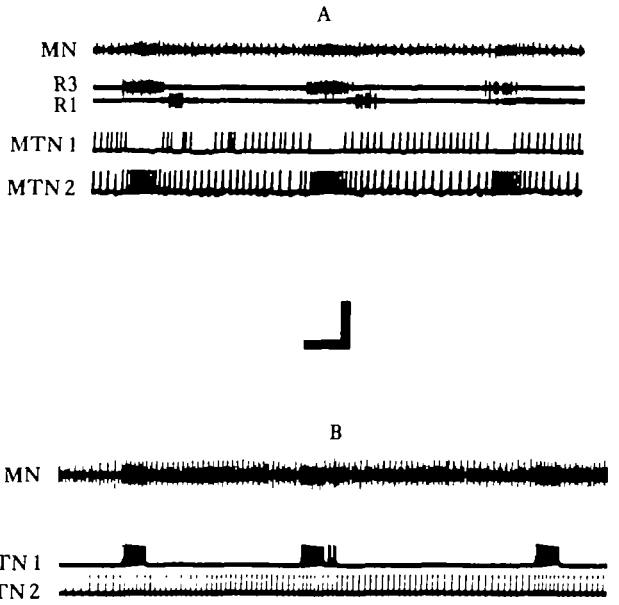

Fig. 14. Comparison of activity, recorded intracellularly in two MN motoneurones (MTN), that was evoked during (A) a primary pattern in an intact buccal-cerebral preparation and (B) tonic CBC stimulation in an isolated brain. Notice the change in sign of input to motoneurone 1, from (A) to (B) during the MN bursts. Same motoneurones in (A) and (B). Calibration: 50 mV, 6 s.

that the latency of brain output to buccal activity remains constant during variation in the cycle period (Cohan, 1980). The major conclusion is that the rhythmic, coordinated activity in the brain and buccal ganglia of *Pleurobranchaea* arises from oscillatory circuits (or neurones) that are located only in the buccal ganglia.

The original proposal of distributed oscillators was based on the observation that tonic stimulation of a ganglionic nerve root led to rhythmic output from other roots of the ganglion. This test has been used traditionally to determine the presence of oscillators in deafferented nervous systems (Hughes & Wiersma, 1960). Our findings show, however, that the cyclical activity that is produced by such tests in *Pleurobranchaea* may be attributable to an artifact by which tonic stimulation of axons produces bursts of action potentials in the axons themselves rather than activating a central oscillator in the ganglion that they innervate.

The remarkable ability of axons to produce bursts of activity when they are tonically stimulated has also been observed in dorsal root fibres of cats (Barron & Matthews, 1935) and sciatic nerve fibres of the frog (Newman & Raymond, 1971). It has been proposed that variations of spike thresholds can give rise to intermittent conduction in which action potentials do not follow each stimulus pulse. Under appropriate stimulus conditions it is possible that cyclical variation in the threshold can delete sequences of action potentials such that the tonic stimulation is converted into phasic bursts of activity (Raymond & Pangaro, 1975; Raymond, 1979). Such oscillatory properties of spike thresholds may be generally applicable to nerves, and, therefore, caution should be taken in attributing rhythmic output to a ganglionic oscillator when the criterion for rhythmicity is based on tonic stimulation of the connectives.

Although it has not been extensively documented, the functional organization described here for *Pleurobranchaea*, whereby a centralized oscillator provides the drive for distributed motor output may be a common feature in the gastropod molluscs. It has been found recently that transection of the CBC during feeding rhythms in *Aplysia* causes the cessation of brain output but not of buccal ganglion output (B. Jahan-Parwar, personal communication), much as the activity of the brain of *Pleurobranchaea* relies on activity in the buccal ganglion. And, as in *Pleurobranchaea*, it has been suggested that neurones in the buccal ganglion of *Tritonia diomedia* send axons to the brain where they may synapse on motoneurones involved in rhythmic movements of buccal structures (Willows, 1978). Interneurones in the buccal ganglion that project to the brain in *Pleurobranchaea* provide both the drive and pattern of activity for the rhythmic motor patterns that are produced by the brain.

It has earlier been assumed that all buccal interneurones which project to the brain of *Pleurobranchaea* carry 'efference copy' (EC) or 'corollary discharge' (CD) information to coordinate activity in the nervous system (Davis *et al.* 1973, 1975). While it may be possible loosely to categorize these interneurones as EC or CD cells, the broadness of these terms as previously used in *Pleurobranchaea* does little to assist us in understanding their functions, especially since these cells are functionally diverse (see Cohan & Mpitsos, 1983). Moreover, these terms seem to imply a functional role which the definition itself may not warrant. Therefore, we have applied the term of buccal-cerebral interneurone (BCI) to these cells which focuses on their anatomical properties and delays decision about their function as a class of neurones until more is known about them.

The authors gratefully acknowledge the help of Dr A. D. McClellan in planning these experiments and Drs A. G. M. Bulloch and D. Barker for helpful criticism of the manuscript. This work was supported by NSF grant BNS 76-8123, AFOSR grant 82-0043A, and PGR grant to G.J.M. and a Sloan Foundation Fellowship and NIH predoctoral fellowship HD 07104-03 to C.S.C.

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