J. exp. Biol. 108, 471–475 (1984)
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RHYTHMIC AND BILATERALLY COORDINATED MOTOR ACTIVITY IN THE ISOLATED BRAIN OF *PLEUROBRANCHAEA*

BY J. A. LONDON* AND R. GILLETTE

Department of Physiology and Biophysics, University of Illinois, 524 Burrill Hall, 407 S. Goodwin, Urbana, Illinois 61801, U.S.A.

Accepted 8 August 1983

Rhythmic ingestive behaviour in the predatory marine snail *Pleurobranchaea* is characterized by cycles of protraction and retraction of the buccal mass, during which food material is taken into the buccal cavity and swallowed (Davis & Mpitsos, 1971). Fictive feeding is demonstrable in the isolated nervous system; the rhythmic motor programme appropriate to feeding behaviour can occur spontaneously or can be elicited by stimulation of different populations of 'command' neurones (Davis *et al.* 1975; Gillette & Davis, 1977; Croll & Davis, 1981; Gillette & Gillette, 1983). Oscillatory motor output is most striking in the isolated buccal ganglion; however, cyclic activity has been recorded in the brain (the cerebropleural ganglion) when it was isolated from the buccal ganglion by cutting the cerebrobuccal connectives (CBCs) (Davis, Siegler & Mpitsos, 1973). Such cyclic activity was recorded in the nerves of the brain while the CBC was stimulated by repetitive electrical shocks. These and other results were interpreted as indicating that oscillators existed both in the brain and the buccal ganglion. Interneurones were identified in both the brain and buccal ganglion which appeared to coordinate the oscillators *via* axons in the CBCs (Davis *et al.* 1973; Gillette & Davis, 1977; Gillette, Kovac & Davis, 1978, 1982).

The interpretation of distributed but coupled oscillators in the brain and buccal ganglion has recently been rejected by Cohan & Mpitsos (1983*a*,*b*). These authors presented results of experiments from which they concluded that rhythmic brain activity is entirely dependent on rhythmic activity in ascending fibres from the buccal ganglion. Essentially, they found that cyclic motor activity recorded in the brain nerves disappeared when CBC conduction was blocked, and rather neatly demonstrated that tonic stimulation of isolated CBC segments by repetitive shocks could cause rhythmic spike activity instead of tonic output. This last observation provided an alternative explanation for the cyclic brain activity caused by tonic CBC stimulation: entrainment of postsynaptic cells occurs by the anomalously cyclic CBC output. In addition, Cohan & Mpitsos (1983*b*) found that cutting the buccal ganglion commissure, which desynchronized the rhythms of the two buccal ganglion lobes, caused desynchronization of the bilateral motor rhythm recorded in the brain nerves. This led them to conclude that the rhythmic motor activity in the two halves of the brain is coordinated unilaterally by ascending fibres from the buccal ganglion, and not through the brain commissure.

* Present address: Yale University School of Medicine, Department of Physiology, 333 Cedar St, P.O. Box 3333, New Haven, CT 06510, U.S.A.

Key words: Motor activity, *Pleurobranchaea*, molluscan feeding.

We have re-examined these issues in order better to characterize the neural organization of feeding behaviour in *Pleurobranchaea*. In this report we demonstrate rhythmic activity occurring spontaneously in the brain when the buccal ganglion was not attached. This activity was recorded from both sides of the brain and the activity of each side appeared phase-locked. Furthermore, in quiescent preparations rhythmic activity could be initiated by application of a food substance (squid homogenate, SH), to the animal's oral veil.

Pleurobranchaea californica (30–120 g) were obtained from Pacific Bio-Marine (Venice, CA) and maintained on a 12 h light/dark schedule in a closed artificial seawater (ASW, Instant Ocean) system at 12–15 °C. An isolated brain-oral veil preparation incorporating chemosensory structures was made from animals that exhibited feeding responses. The preparation consisted of the oral veil, rhinophores, mouth and the brain excised from an unanaesthetized animal. All nerves and connectives, except for the large and small oral veil nerves were severed. In one experiment, the rhinophore nerves were left intact, and the oral veil nerves were severed. The preparation was placed in a water-jacketed Lucite chamber, immersed in buffered ASW (pH 7·5) and maintained at 11–13 °C. The brain was pinned to a Sylgard (Dow) block fastened to the floor of the chamber to stabilize the brain for recording. Standard electrophysiological recording techniques were employed. For intracellular recording, 3 M-KCl-filled microelectrodes led to high impedance amplifiers (WPI M4-A). For each extracellular recording, the cut end of a nerve was drawn into the end of a suction electrode which led to a differential amplifier. At least 45 min was allowed for recovery from the effects of electrode placement before experimental measurements were made. All signals were displayed on an oscilloscope and permanent records were made using a Brush 220 chart recorder.

Spontaneous rhythmic activity was observed in all of seven preparations examined. The rhythmic activity consisted of bursts of axon spikes recorded extracellularly from the CBCs (Fig. 1A). Rhythmic activity occurred simultaneously in both CBCs. The frequency of spontaneous bursts was 2–3 cycles per minute which is typical of the frequency of activity of a deafferented preparation (Siegler, 1977).

In one preparation, spontaneous rhythmic activity was recorded intracellularly from a putative oral veil motoneurone (Fig. 1B). The activity in this neurone slightly preceded the burst activity of the CBC, suggesting that the activity in the brain was not driven by the activity in the CBCs. The activity in this motoneurone was phase-locked with that of the CBC; spontaneous phase delays in the activity of this cell were coincident with delays in burst activity of the CBC. This activity continued for several minutes (Fig. 1B).

Rhythmic activity could be induced in quiescent preparations ($N = 3$) by applying SH to the animal's oral veil (Fig. 1C), but not by egestive stimuli (ethanol or soap solution) or noxious pinches. Such food stimuli also elicited extension of the oral veil and flaring of the sensory papillae, as are typical of appetitive feeding behaviour (Gillette & Gillette, 1983). It is possible that the neural oscillator in the brain of *Pleurobranchaea* might be involved in more than one type of behaviour, as is the case for the oscillator in the buccal ganglion which mediates both ingestion and egestion (McClellan, 1980; Croll & Davis, 1981). Such systems may exhibit metastable coordination (Ayers & Davis, 1977) such that different sensory inputs can effect a

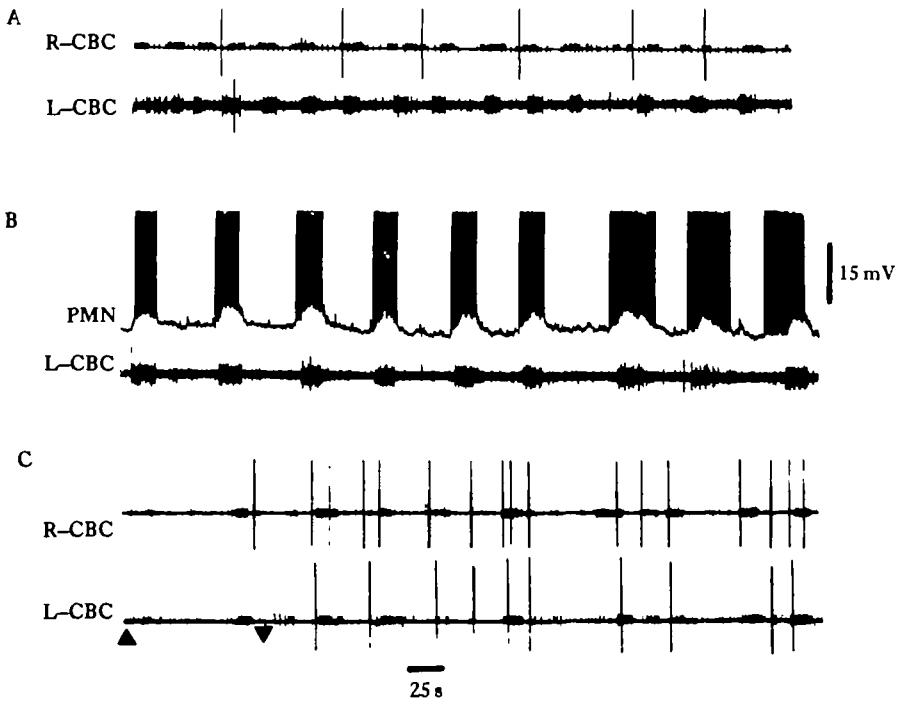

Fig. 1. Rhythmic activity in the isolated brain. (A) Extracellular recordings of each CBC indicating that the brain can generate spontaneous, rhythmic activity. In addition, this rhythmic activity is coupled between both sides of the brains as reflected in the simultaneous or near-simultaneous bursts of activity of both CBCs. (B) Simultaneous activity of a putative motoneurone and the ipsilateral CBC in an isolated brain. The top trace is an intracellular recording of the spontaneous activity of a putative small oral veil motoneurone (PMN). The bottom trace shows an extracellular recording of the CBC ipsilateral to the PMN. Note that the PMN activity precedes the activity in the CBC, suggesting that this cellular activity is the result of some interneuronal oscillator in the brain and not due to some spontaneous activity in the CBC. The tops of the action potentials are clipped. (C) Rhythmic activity induced in the isolated brain of a quiescent preparation as a result of food application. Extracellular records of the left and right CBC of a preparation show no rhythmic activity at first. However, during squid homogenate application (between arrows) rhythmic activity is induced. The activity of both sides can be seen to be strongly coupled.

dynamic reorganization of the oscillator to produce different types of outputs. In this case the nature of the stimulus and the behavioural response suggest that this rhythm might be appropriately characterized as fictive feeding rather than egestion.

SH-induced activity occurred with a frequency of 2–4 cycles per minute, a frequency which is relatively low for feeding in the intact animal and which may reflect a less robust nature of the brain oscillator compared to the buccal oscillator. Bursting activity recorded in the CBCs usually continued for some minutes after cessation of SH application. This indicates that appetitive chemosensory input is adequate to drive an oscillator in the brain, and that the oscillator can function in isolation from that input or from input from the buccal ganglion. In two preparations, severing the remaining nerves connecting the brain to peripheral structures did not result in cessation of the rhythmic activity (data not shown), further indicating the presence of a true central pattern generator.

Records like that of Fig. 1A also show that the cyclic activity generated by the brain oscillator is bilaterally coordinated. Bilateral coordination is also present in the records of Fig. 1C for which activity was stimulated by SH application. The preparation used in Fig. 1C had the rhinophore nerves intact and the oral veil nerves severed. The larger units that are repetitively active in these records are a bilateral pair of identified neurones, the metacerebral giant neurones (MCGs; Gillette & Davis, 1977). These neurones are not directly coupled to each other, but spike activity in these neurones is often synchronous in both the isolated nervous system and in the whole animal (Gillette & Davis, 1977). The spike activity of the MCGs also shows synchrony in the isolated brain-oral veil preparation (Fig. 1C). MCG synchrony continued when the rhinophore nerves were severed (data not shown). Fig. 2 also shows this coupling in a histogram plotting the number of occurrences of an MCG spike in one CBC in the intervals on either side of the MCG spike event in the contralateral CBC. The data for this histogram were taken from a preparation with oral veil nerves intact and rhinophore nerves severed. A Chi square (χ^2) analysis indicates that these events were not random [χ^2 (3) = 22·0, $P < 0{\cdot}001$].

One bilateral population of interneurones which is capable of coordinating cyclic motor activity between the brain hemi-ganglia is the Interneurone 2 group (Int-2) (London & Gillette, 1981, 1984; Kovac, Davis, Matera & Croll, 1983). These interneurones, which fire cyclically during feeding behaviour, excite or inhibit many brain neurones of the feeding network. Among the follower cells of the Int-2s are the MCGs, which they drive *via* potent, monosynaptic and excitatory connections. The Int-2s of one bilateral group make effective, reciprocal and excitatory synapses with the contralateral Int-2 group *via* commissural axons. It is likely that the Int-2s are an important part of the bilateral coordinating mechanisms.

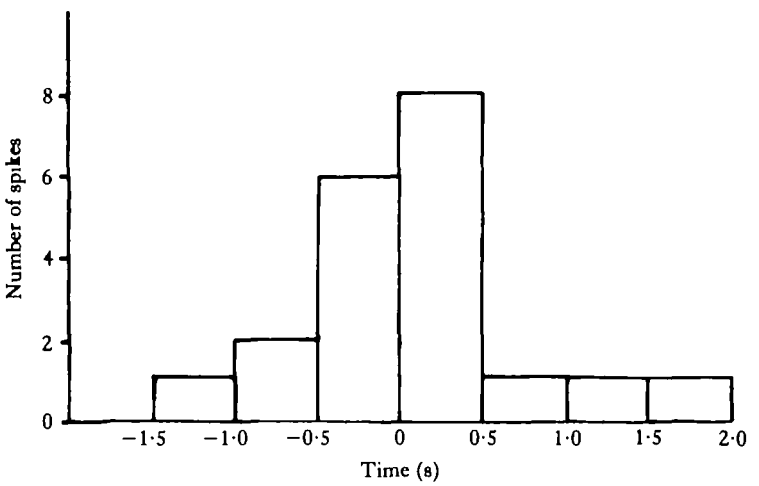

Fig. 2. An analysis of the coupling of MCG activity. This histogram plots the number of times one MCG spike occurred in a specific time interval centred around the contralateral MCG spike during feeding. It can be seen that the spikes occur nearly simultaneously, 14 of the 20 spikes of one MCG occurred within 1 s of the contralateral MCG spike, indicating a tight coupling of activity of the two cells.

The reasons that Cohan & Mpitsos failed to observe oscillatory activity or inter-hemispheric coupling in the isolated brain are not clear. Possibly their laboratory's methods of anaesthesia prior to dissection, $MgCl_2$ and Nembutal injection (McClellan, 1980), cause lasting depression of excitability in the nervous system. Prolonged Nembutal exposure has been shown to result in irreversible inhibition of neurones in some invertebrate preparations (Sato, Austin & Yai, 1967).

The oscillatory central pattern generation of feeding behaviour in *Pleurobranchaea* is consistent in its structure with the general features of other central pattern generators (cf. Selverston, Russell, Miller & King, 1976; Kristan, 1980; Robertson & Moulins, 1981). As with other systems, the oscillatory characteristics of this system are distributed at multiple levels: the intrinsic properties of the neurones, the connectivity among the elements and the coupling among separable oscillating networks.

This research was supported by National Science Foundation Grant BNS 7918329.

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