BURST ACTIVITY OF THE BUCCAL GANGLION
OF *APLYSIA DEPILANS*

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INTRODUCTION

In an earlier paper (Rose, 1971*b*) it was shown that bursts of impulses occur in the buccal nerves of the isolated buccal mass of *Archidoris* during induced feeding movements. Kling & Szekely (1968) and Rose (in preparation) have predicted more complex output patterns in theoretical analyses of small networks of bursting neurones. This paper describes such a complex burst sequence, which can be elicited from the buccal ganglion of *Aplysia depilans* by injection of food into the buccal cavity of the isolated buccal mass. Each sequence involves 1–4 cycles of burst activity, having a total duration of up to 110 sec. It is interesting that Dorsett, Willows & Hoyle (1969) have described a similar burst sequence involving neurones in the pedal ganglion of *Tritonia gilberti* during the swimming escape response. Their preparation showed 2–8 cycles of burst activity which lasted about 55 sec. In another publication (R. M. Rose, in preparation) it will be shown that the burst periods of both the *Tritonia* and *Aplysia* sequences can be fitted by solutions of modified forms of van der Pol's equation for a relaxation oscillation (van der Pol, 1926).

Recent work by Kandel *et al.* (1967, 1968, 1969) on the abdominal ganglion, and Gardner (1969) on the buccal ganglion of *A. californica*, and also Levitan, Segundo & Tauc (1970) on the buccal ganglion of *Navanax*, has shown that it is possible to define connexions between identified cells by physiological means. It seems possible that the buccal ganglion of *A. depilans* is a preparation in which the connexions between cells could be specified, the output patterns could be characterized and related to radula movements, and these patterns could also be described mathematically. These appear to be the main steps involved in relating nervous activity and behaviour.

METHODS

Preparation

Aplysia depilans was used exclusively. Initial experiments were carried out at Arcachon in September 1968, and subsequently for the whole of June 1969, at Naples and the whole of September 1970, at Arcachon. Animals were kept in running sea water at room temperature. Each animal was pinned out in a dissecting dish and the digestive system was exposed by a cut through the body wall. The buccal mass and stomach were removed by cutting posterior to the stomach, and by cutting all nerves from the brain except the cerebral and buccal nerves. The buccal mass was then

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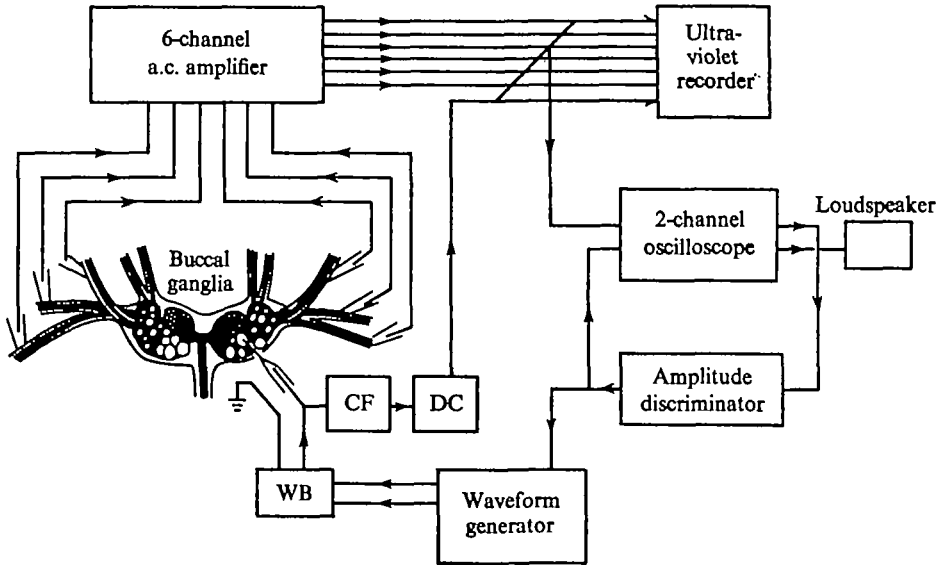
Fig. 1. Recording arrangement. The simultaneous activity of six nerves in amplified (AC) and displayed on an ultraviolet recorder. One of these channels is selected and the activity displayed on the upper beam of the oscilloscope. The amplitude discriminator is adjusted to select a particular burst in this nerve, the output pulse of which is used to trigger the waveform generator. The output waveform is applied to a bridge circuit (WB) to depolarize a cell impaled with the micro-electrode. The activity of this cell is fed into a cathode follower (CF) and amplified (DC) for display on the seventh channel of the ultraviolet recorder.

transferred to a dish of sea water at room temperature and the buccal ganglia were exposed by cutting through the thin connective tissue and aorta overlying the ganglia and were pinned out on a small wax-covered table. Feeding movements were induced in the isolated buccal mass by injecting homogenized sea-weed (*Ulva*) into the buccal cavity. A rubber tube was inserted through the oesophagus, a ligature was tied to hold it in place, and the sea-weed homogenate was injected through the tube using a syringe. Each animal was cooled to 5–10 °C for about half an hour prior to the experiment, and each injection of sea-weed was made 10–20 min after a previous injection to allow time for recovery. Generally the longer the interval between injections the more prolonged was the response.

Recording technique (Fig. 1)

Extracellular recordings were made from up to six nerves simultaneously using a technique similar to that described previously (Rose, 1971 b). The activity of the six nerves was recorded with suction electrodes mounted on six separate manipulators of universal joint construction (Grass BS-3). The recording leads were fed into six a.c. amplifiers and then into six channels of an ultraviolet recorder (Southern Instruments, series 10–200). Individual units from any of these six nerves were selected using an amplitude discriminator (Plumb, 1965). This was done by selecting the output of one of the amplifiers and taking it into the upper channel of a Tektronix 502A oscilloscope, and then into the amplitude discriminator. The level was set to select a given unit and the output pulses of the amplitude discriminator were displayed on the lower channel of the oscilloscope. The first output pulse of the selected burst was then used

to trigger a waveform from a Servomex type LF51 low-frequency waveform generator. This waveform was applied across a conventional bridge circuit to produce a depolarizing waveform in another cell which was impaled with a micro-electrode. The use of the amplitude discriminator to trigger the waveform generator provided a means of depolarizing a given cell at the same time as a burst occurred in one of the buccal nerves recorded extracellularly. The depolarizing waveform produced a burst in the impaled cell, and interactions between this cell and the selected burst were observed.

Intracellular recordings were made with glass micro-electrodes filled with 3 M-KCl, and having a resistance of 5–10 MΩ. A field-effect transistor input probe was used, the output of which was recorded on the seventh channel of the ultraviolet recorder after d.c. amplification. The ganglion was pinned out carefully prior to penetration using micro-pins placed through the thick connective tissue sheath. In some cases this sheath was cut open with a sliver of razor blade, but an alternative technique was to leave the thick connective tissue intact and use an electro-mechanical device to help penetration by the electrode. This consisted of a high-impedance loudspeaker with the micro-electrode firmly attached to the coil, which was activated by a short-duration pulse to punch the electrode through the sheath (Weevers, 1971).

RESULTS

The paired buccal ganglia are each approximately 1 mm in diameter, and are joined to each other by a distinct commissure and to the cerebral ganglia by the paired cerebrobuccal connectives (C). The six buccal nerves have been labelled B1–B6 (Fig. 2). B1 is a median nerve arising from the middle of the transverse commissure, and passes ventrally to the base of the radula sac where it enters the buccal musculature. Three nerve trunks originate laterally from a point opposite the commissure. The largest is the cerebro-buccal connective (C). The next largest is the ventral buccal nerve B2/3, which passes round the side of the buccal mass and divides into the two nerves B2 and B3. The other nerve leaves the base of the cerebro-buccal connective and runs dorsally to supply the upper part of the buccal mass, and this has been labelled as nerve B4. Nerves B5 and B6 arise from lobe 2 (see below) of the buccal ganglion and supply the salivary glands and oesophagus respectively. Nerves from the left or right buccal ganglion will be preceded by L and R respectively, so that RB2 is the nerve B2 on the right-hand side.

For descriptive purposes the buccal ganglia have been divided into three lobes which have been labelled L1–L3 in Fig. 2. Eighteen cells can be seen on the dorsal surface and a further nine on the ventral surface of lobe 1. These are numbered in order of decreasing size in Fig. 2, although this description cannot be supported by physiological identification of the cells at this stage. Lobe 2 contains 8–10 medium-size cells and may be equivalent to the gastro-oesophageal ganglion of other molluscs, since it has a clearly defined outline and nerves B5 and B6 originate from it to supply the salivary glands and oesophagus. Lobe 3 contains small cells, and compared with the nudibranch species *Archidoris* (Rose, 1971a) this region of small cells is much larger in *Aplysia*.

In a previous paper (Rose, 1971b) the burst activity of the buccal ganglion of *Archidoris* was divided into two main types.
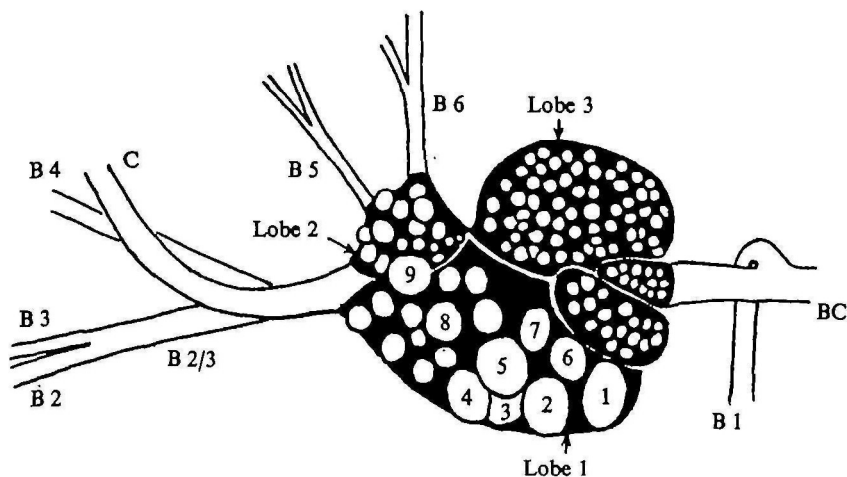
Fig. 2. Diagrammatic representation of the dorsal surface of the left buccal ganglion of *Aplysia depilans*. Three lobes have been labelled. The largest cells in lobe 1 have been numbered 1–9. Lobe 2 contains medium-sized cells, and lobe 3 numerous small cells. There are six buccal nerves (B1–B6), together with the left cerebro-buccal connective (C) and the buccal commissure (BC).
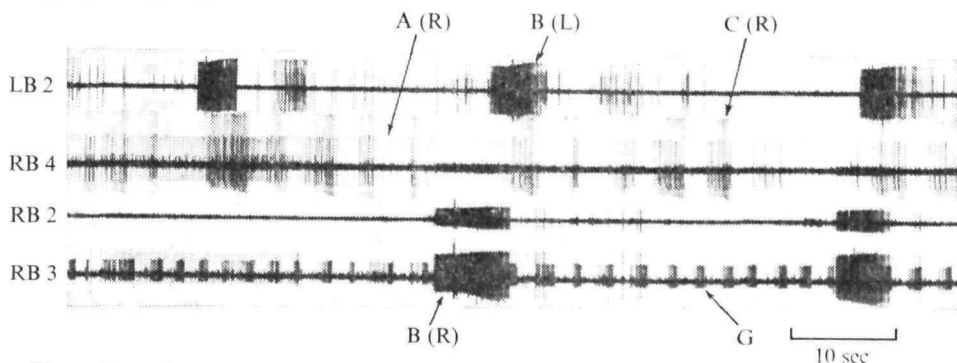

Fig. 3. Four-channel extracellular recording showing long-duration (A), medium-duration (B), and short-duration (C) regularly firing bursts.

(A) Regularly firing bursts, which were repeated at regular intervals and generally had no fixed phase relations to other bursts.

(B) Sequentially firing bursts, which were only seen during feeding movements and had fixed phase relations to other bursts.

The burst activity of the buccal ganglion of *A. depilans* will also be discussed under these headings, although strictly speaking these categories grade into each other.

A. Regular burst activity

Extracellular recording

Examples of regular burst activity recorded extracellularly from the buccal nerves in four preparations are shown in Figs. 3–5. Individual units have been labelled as A–I and bursts A, B, and C have been identified in different preparations on the basis of similar spike amplitude, burst duration, and spike repetition frequency. Bursts recorded on opposite sides have been distinguished by the letters L (left) and R (right). There are three main types of burst. (*a*) Long-duration bursts (A, H, and I) lasting
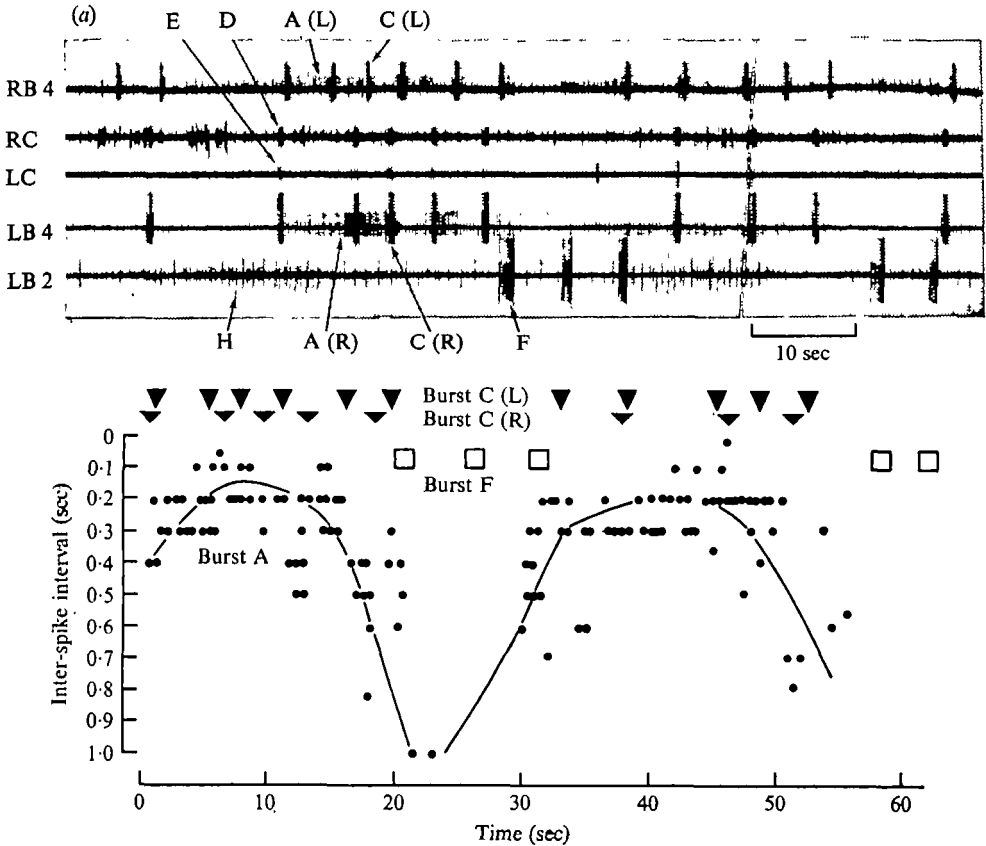
Fig. 4 (a) Five-channel extracellular recording from three buccal nerves and the paired connectives. The short-duration bursts C, D, and E discharge simultaneously (nerves RC, LC, LB4), and tend to fire in groups of 3-5 short-duration bursts separated by a long interval.

(b) Inter-spike interval change for the long-duration burst A shown in (a). The change is approximately sinusoidal, and unit C bursts (black triangles) tend to occur when burst A is discharging at high frequency. Burst F (open squares) fires when burst A is discharging at a low frequency.

> 20 sec with low spike repetition frequency (1-10 pulses per second). (b) Medium-duration bursts (B) lasting 5-10 sec with high spike repetition frequency (10-20 p.p.s.). (c) Short-duration bursts (C-G) lasting > 5 sec with a high spike repetition frequency (5-15 p.p.s.).

These *recordings show evidence of synchrony between bursts on the same side* and on opposite sides, and a tendency for groups of bursts to develop. Thus for units A, B, and C, partner bursts on opposite sides occur approximately simultaneously (although not exactly synchronously). Fig. 5b is intermediate between regular and sequential burst activity and it is possible to identify bursts A and C as bursts $\alpha 1$ and $\alpha 2$ to be described later in burst sequences in nerve B4. Close synchrony occurs between the short duration bursts C, D, and E on the same side, and these bursts also tend to occur in groups of 3-6 bursts separated by an interval of about 20 sec. In Fig. 4b the inter-spike interval change for burst A in Fig. 4a is shown to be approximately sinusoidal, and a grouping of C/D/E bursts (black triangles) occurs near the point where burst A is firing at maximum frequency.
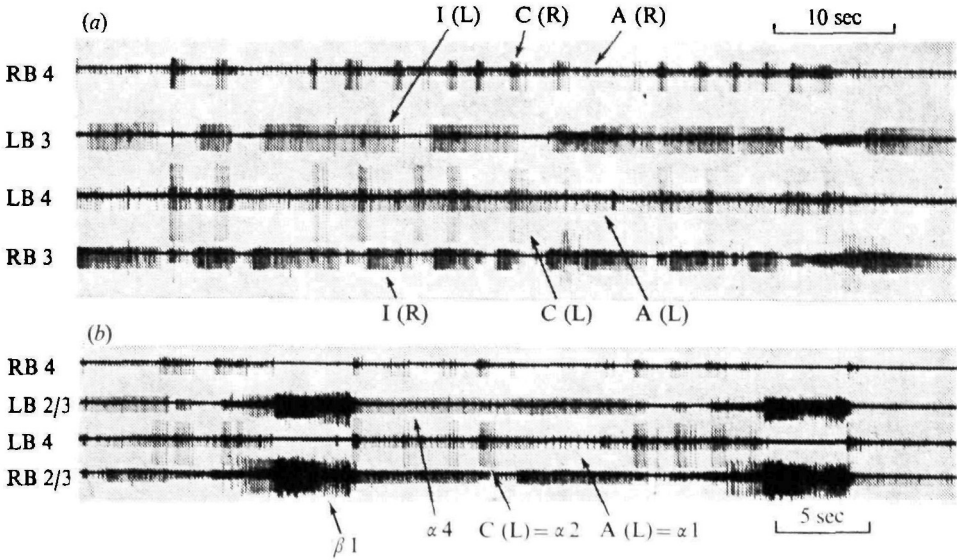
Fig. 5. (a) Example of partner bursts in symmetrically placed nerves. Three units (A, C and I) discharge on each side.

(b) Activity which is intermediate between regular and sequential burst types. This recording has been used to identify bursts A and C as units $\alpha 1$ and $\alpha 2$ of sequential burst activity (see Fig. 12).
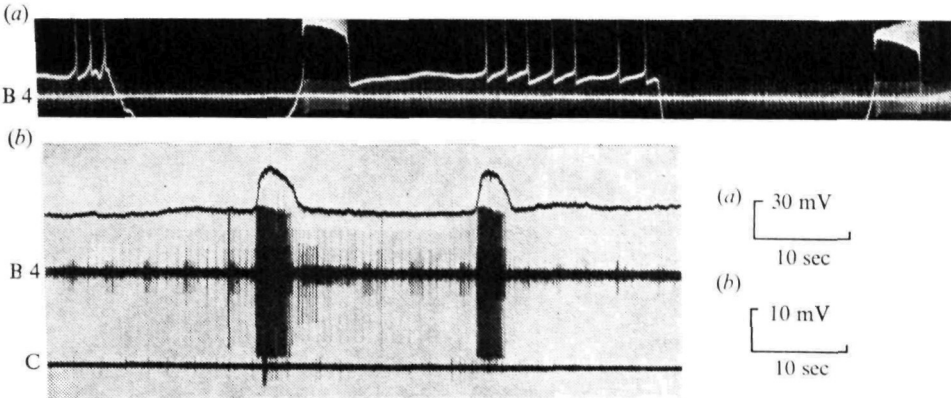

Fig. 6. (a) Simultaneous intracellular recording from an unidentified cell in lobe 1 and extracellular recording from nerve B4. A prolonged potential change occurs in the cell each time it is depolarized. A burst occurs in nerve B4 simultaneous with these potential changes.

(b) Simultaneous intracellular recording from another unidentified cell in lobe 1, and the activity of nerves B4 and C recorded extracellularly. A high-frequency short-duration burst occurs in nerve B4 simultaneous with a depolarizing waveform in the cell.

Intracellular recording

A detailed intracellular study of regularly firing bursts has not yet been made but two interesting examples are shown in Figs. 6 and 7 for two unidentified cells in lobe 1. In Fig. 6a the cell was hyperpolarized on two occasions, and then depolarized. In each case an initial spike-like potential is prolonged near its peak for 4–5 sec, and then switches off and is followed by a long refractory period. Simultaneous with this prolonged potential a burst occurs in the ipsilateral nerve B2. This potential may be similar to the prolonged action potentials described for neurones of *Helix* and *Planorbis*
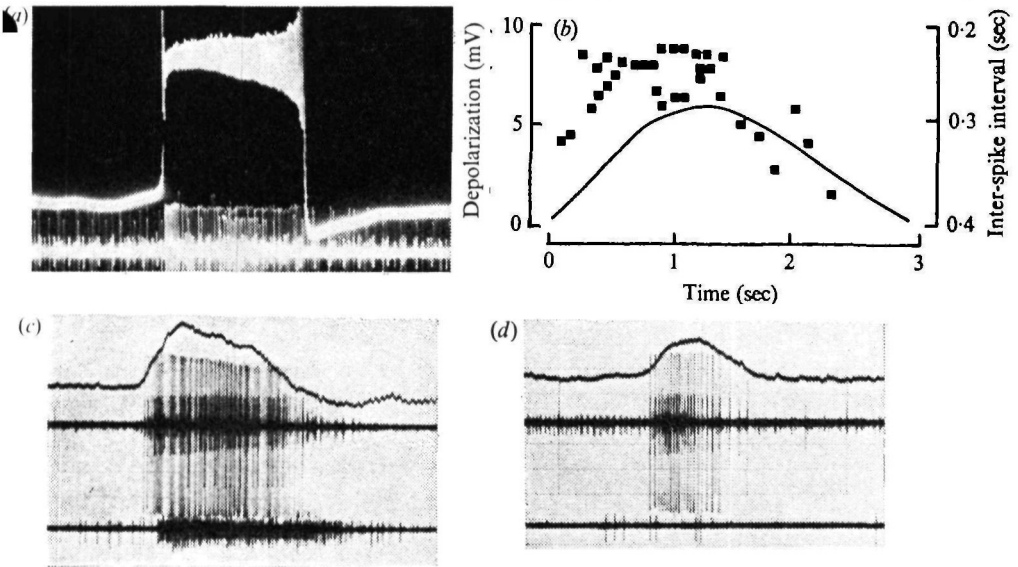
Fig. 7. (a) Detail of the cell shown in Fig. 6(a). The membrane potential oscillations gradually increase in amplitude, and then are suddenly terminated. A prolonged undershoot follows. A burst occurs simultaneously in nerve B4.

(b) Plot of inter-spike interval change (black squares) together with membrane potential change of the burst shown in (d).

(c) and (d) Further examples from the cell shown in Fig. 6(b) at higher film speed. The membrane potential change is of greater amplitude for the high-frequency burst in (c) compared with the low-frequency burst in (d).

in the presence of additional barium ions in the extracellular medium (Kostyuk, 1967). The potential changes are shown in more detail in Fig. 7a. It is difficult to decide whether there is a membrane oscillation or repolarizing spike-like potentials occurring at very high frequency, but it does appear that the mechanism is concerned with the generation of a high frequency of discharge, and that extracellularly recorded spikes may be 1:1 with membrane potential charges. There is a prolonged hyper-polarization at the end of the burst, although the initial undershoot is similar in magnitude to that of a normal action potential in this cell.

Fig. 6b shows a cell in which a positive-going waveform occurred simultaneous with a high-frequency regularly firing burst in nerve B4. This waveform was below the threshold for initiating an action potential. In Fig. 7c and d two further examples from the same cell are shown at higher film speed. The membrane potential change is of greater amplitude and longer duration when a long burst occurs simultaneously (Fig. 7c) compared with a short burst (Fig. 7d). The inter-spike interval change of the burst in nerve B4 closely parallels the membrane potential change in every case analysed. An example is given in Fig. 7b which shows a tracing of the membrane potential change in Fig. 7d, together with the associated interspike interval change of the burst in nerve B4. These recordings suggest that the bursting unit in nerve B4 is driving the cell recorded on the upper channel, the unusual feature being the absence of action potentials in spite of a large depolarizing waveform.
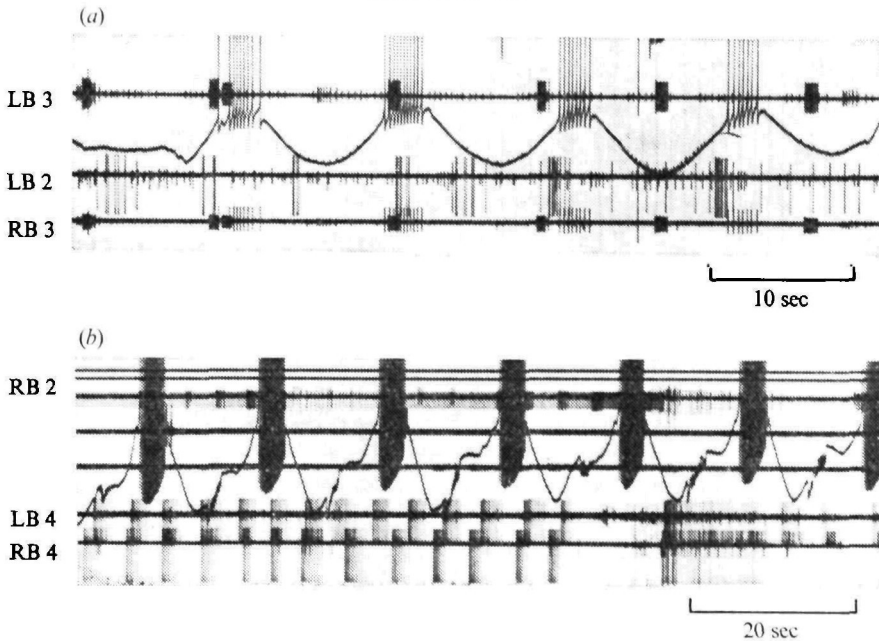
Fig. 8. Effect of passing a depolarizing sine wave into a cell in two different preparations. In both cases the burst has no effect on regular burst activity recorded simultaneously in three buccal nerves. In (b) there is a burst of inhibitory potentials on the rising phase of each sine wave.

Injected waveforms

Preliminary recordings were made to discover whether burst activity produced in a given cell by a depolarizing input waveform had any effect on spontaneously active regularly firing bursts. Bursts were either evoked at regular intervals, or alternatively the impaled cell was depolarized simultaneously with a burst recorded extracellularly in a buccal nerve.

Fig. 8 shows two examples of four-channel recordings made in two different preparations, in which a low-frequency sine wave was passed into an unidentified cell in lobe 1 in each case. In both cases the bursts produced in the impaled cell had no effect on regularly firing units. However, it should be noted that a burst of inhibitory post-synaptic potentials (ipsps) always occurs on the rising phase of each depolarizing sine wave in Fig. 8b, suggesting that another cell has become phase-locked to this burst. Bursts of ipsps were not recorded in this cell when the shift in membrane potential was not imposed.

Two further examples are given in Fig. 9 which show the effect of depolarizing cells at specific points in time. A given burst was selected from an extracellular recording made from a buccal nerve, using a spike-amplitude discriminator (Fig. 1). The output pulse of the discriminator was then used to trigger a square wave which was used to depolarize the impaled cell. In both cases bursts were produced in the impaled cell, which were synchronous with the selected burst recorded extracellularly. In Fig. 9a, a depolarizing square wave was triggered from a unit recorded on the upper channel (arrowed). The short burst produced by the depolarization does not have any constant effect on the selected burst or on the burst recorded on the lower two channels. Simi-
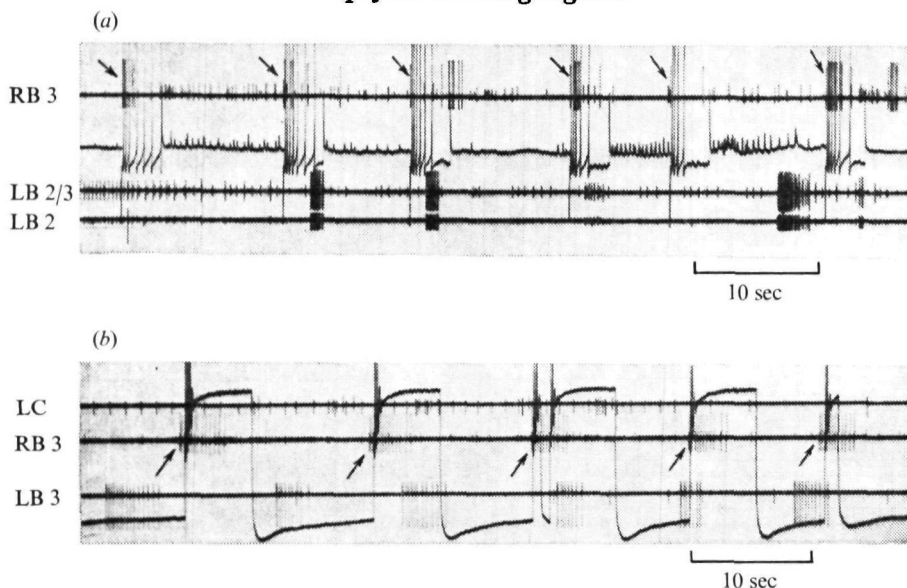
Fig. 9. Examples from two different preparations to show the effect of causing one cell to fire a burst simultaneous with a burst in another. In each case the activity of three nerves is shown, recorded extracellularly, together with the activity of another cell recorded intracellularly.

(*a*) A square pulse of 3 sec duration is triggered from the burst in nerve RB 3 (arrows, upper channel). (*b*) A square pulse of 5 sec duration is triggered from a burst in nerve RB 3 (arrows, second channel).

larly in Fig. 9*b*, the square wave was triggered from the bursting unit recorded on the second channel (arrowed), and the short-duration burst which was produced had no effect either on this original bursting unit or its partner cell in nerve LB 3 recorded on the third channel.

B. *Sequential burst activity*

Injection of *Ulva* homogenate into the buccal cavity of the isolated buccal mass produces a quite different type of activity from that discussed in section A. In most cases a sequence of overlapping bursts is initiated, lasting up to 110 sec in some instances. Occasionally this sequential activity occurs spontaneously without injection of food, in which case sequences occur at approximately 5 min intervals separated by periods in which there is very little activity. It was found that the injection of food was most effective when there was little on-going regular burst activity; the animal was therefore cooled prior to the experiment to try to eliminate this activity.

Two terms will be used in the descriptions which follow: (1) Sequence – the total period of activity lasting 30–110 sec, depending on the conditions. Up to 12 bursts have been identified in a given sequence, each burst (or unit) being a train of action potentials of constant amplitude. (2) Cycle – each sequence is divided into 1–4 cycles. A cycle is a period between successive firings of a given burst. In a given cycle all the bursts involved in the sequence usually discharge. Furthermore, the order of bursting is usually fairly constant on each cycle. Each cycle has been labelled as τC, and has also been divided into two periods, τA and τB. During each sequence there are strong contractions of the buccal musculature, including forward and backward movements of the radula, and it has been observed that the τA and τB periods of a cycle correspond
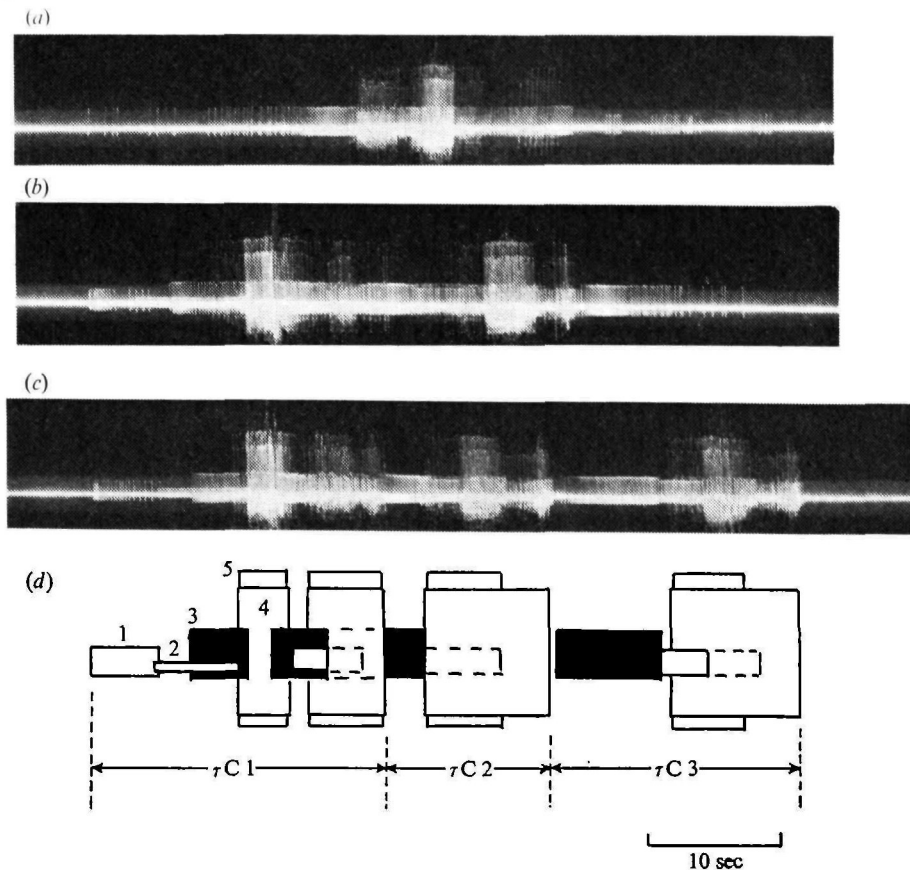
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Fig. 10. Sequential burst activity recorded extracellularly from nerve RB2/3 in a single preparation showing (a) one, (b) two, and (c) three cycles of activity. (d) Representation of the sequence shown in (c). Five units have been identified (1-5), and the three cycles (τC1-τC3) labelled. There is evidence of alternation of activity between burst 3 (shaded black) and bursts 4 and 5 (largest-amplitude bursts).

respectively to these forward and backward movements. But no attempt will be made in this paper to relate burst activity to feeding movements.

Three examples of sequential burst activity recorded with a suction electrode on nerve RB2/3 are shown in Fig. 10. Sequences are shown with one, two, and three cycles of activity. Five units have been identified on the basis of constant spike amplitude and are labelled as 1-5 in Fig. 10d. The three cycles of activity have also been labelled as τC1, τC2, and τC3 in Fig. 11D. Two points of importance are shown in these recordings. First, bursts show some constancy of timing in relation to other bursts for repetitions of the sequence. However, there are variations in cycle time τC, and differences in the timing of bursts on the first cycle compared with later cycles. Secondly, there appears to be an alternation of activity between burst 3 (shaded black in Fig. 10d) and bursts 4 and 5 (largest-amplitude units).

A further example, giving more detail of the sequence, is the 5-channel extracellular recording shown in Fig. 11, which was made from nerves B2 (right and left), B3 (right and left), and B4. The sequence is made up of four cycles of activity (τC1-τC4), each of which has been divided into two periods (τA and τB). Four units
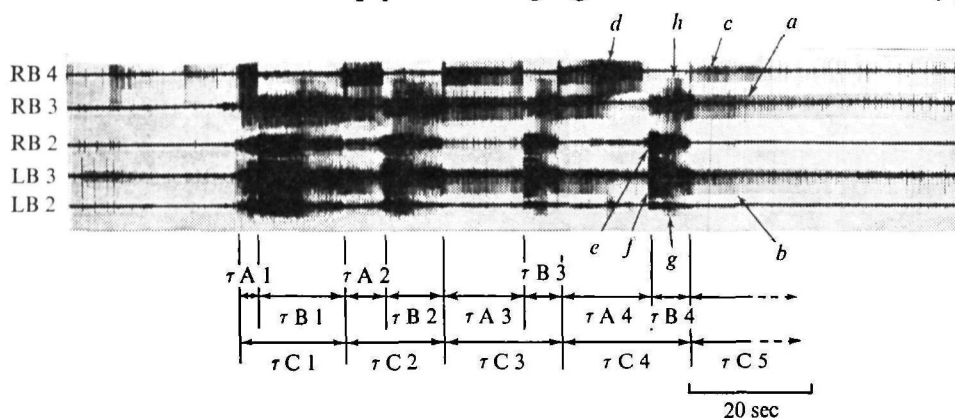
Fig. 11. Simultaneous extracellular recording from five buccal nerves. Four cycles (τC 1–τC 4) have been labelled, and each divided into τA and τB periods. Units *a–d* discharge in each τA period, and units *e–h* in each τB period.

have been identified in each period (*a–d* for period τA, and *e–h* for period τB). In the τA period, units *a*, *b*, and *c* discharge nearly synchronously (but spikes not 1:1) on all cycles. Units *a* and *b* are clearly symmetrical partner cells, since they are recorded in nerve B3 on opposite sides, and discharge for a longer period than unit *c* on the fifth cycle (τC 5). Unit *d* gives a burst of about ten spikes at the beginning of each τA period, which is followed by a silent period, after which it resumes firing. On the fourth cycle (τA 4) unit *d* discharges at a higher frequency than on other cycles, and units *a* and *b* terminate early instead of firing for the whole of the τA period as on previous cycles. This suggests an interaction between units *a/b* and unit *d*. Other examples of this interaction will be discussed later.

The bursts recorded in the τB period consist of two pairs of synchronous bursts (*e* and *f*, and also *g* and *h*), the two pairs being displaced relative to each other. The *e* and *f* burst-pair discharge at a higher frequency than the *g* and *h* burst-pair, and the onset of units *g* and *h* occurs later than that of units *e* and *f*, although all four bursts terminate together. In relation to previous recordings (Fig. 10), it is probable that unit *a* (and partner burst *b*) is the same as burst 3 in Fig. 10, and also that one of the units *e* and *g* corresponds to either burst 4 or 5 of Fig. 10. The main point is that there is again an alternation of activity between units *a* and *e*. Inspection of Fig. 11 suggests that the sequence may be considered as an alternation of two groups of bursts firing in the τA and τB periods.

Detailed analysis of a sequence

In spite of variations in different sequences it is possible to identify the same units in many different preparations. In order to provide a basis for future work it is therefore important to classify the bursts as far as possible. Two representative examples will be considered in detail.

Fig. 12 is a four-channel extracellular recording of a sequence in another preparation, in which the recording paper was run at a higher speed to show details of changes in spike repetition frequency in individual bursts. Recordings were made from nerves B2 (left and right), B3, and B4. There are three cycles of activity (τC 1–3), and nine bursting units have been identified on the basis of spike amplitude, change of spike
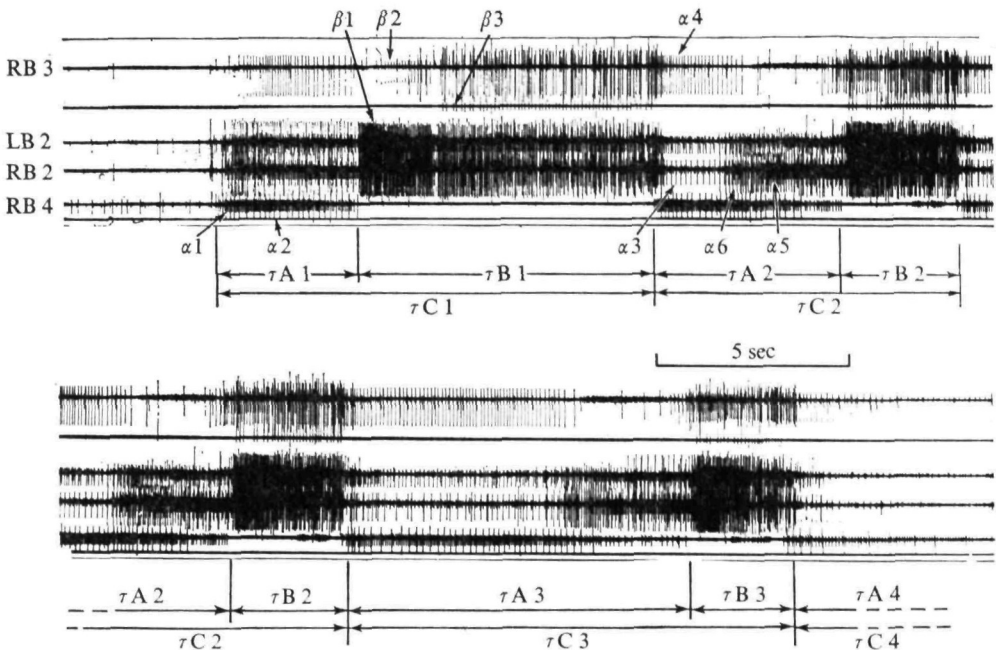
Fig. 12. Continuous 4-channel extracellular recording showing details of a 3-cycle sequence (τC1–τC3). The two recordings are consecutive. Units α1–α6 fire in each τA period, and units β1–β3 in each τB period. There is clear evidence of alternation of bursts in units α1 (lower channel) and β1 (third and fourth channels). Bursts α1–α4 discharge in synchrony, although α4 cuts off early on each cycle.

repetition frequency, and time of burst occurrence. Of these, six fire in the τA period and three fire in the τB period, although one unit (α4) tends to fire in both periods. In Fig. 13 the inter-spike interval changes have been measured for each burst and plotted on a logarithmic scale against time to show any exponential changes. For ease of understanding of the sequence, the bursts have been separated into four groups (which do not relate to each of the four channels in the recordings). The two groups at the top are from the bursts which show the clearest evidence of the alternation of bursts in τA and τB periods, and the lower two groups provide additional information on bursts in the τA period (3rd group) and τB period (4th group). This plot will be used in conjunction with the actual recording in the following description of each unit in the sequence.

(i) τA-period bursts

Six units have been identified in the τA period, and have been numbered α1–α6 in Figs. 13 and 14.

Unit α1 is a small-amplitude burst recorded in nerve B4 (lower channel), whose discharge rate is quite high (2–5 p.p.s.). This burst alternates exactly with unit β1 (large-amplitude unit on 3rd channel). The burst becomes progressively longer on the second and third cycles (τA2 and τA3), and also on the second and third cycles the inter-spike interval change has a characteristic form, with a high initial frequency, which falls slightly, and then rises again, followed by a gradual decline. This has been interpreted as a low-frequency damped oscillation (R. M. Rose, in preparation) and is
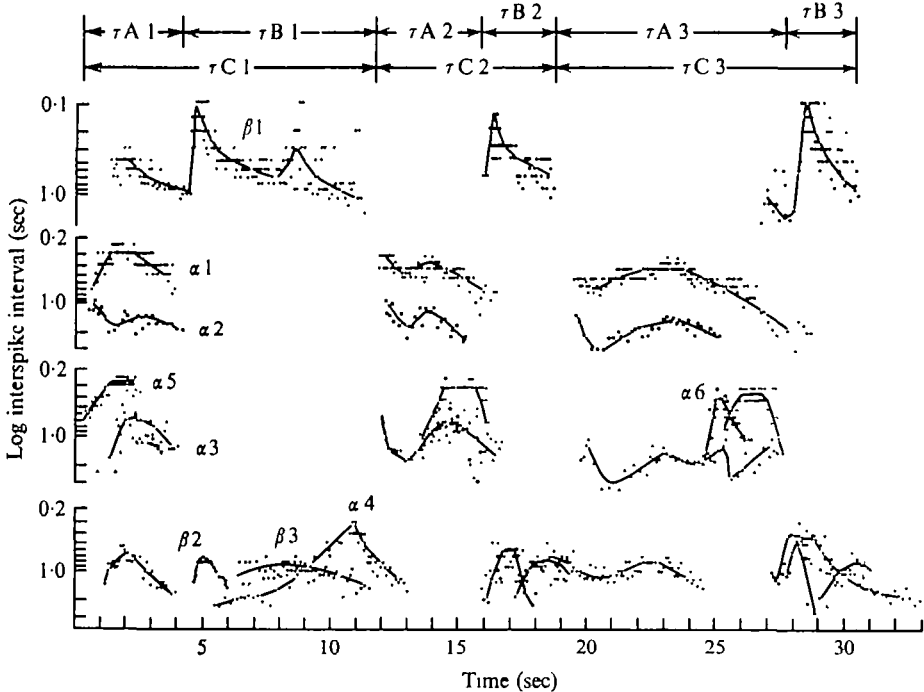
Fig. 13. Inter-spike interval plots of units $\alpha 1$–$\alpha 6$ and $\beta 1$–$\beta 3$ for the 3-cycle sequence shown in Fig. 12. Inter-spike intervals measured by eye and plotted on a logarithmic scale (to show exponential decay in any unit) against time on a linear scale. The bursts have been separated into four groups. (a) Burst $\beta 1$, (b) Bursts $\alpha 1$, $\alpha 2$, (c) Bursts $\alpha 3$, $\alpha 5$, $\alpha 6$, (d) Bursts $\beta 2$, $\beta 3$, $\alpha 4$. Bursts $\alpha 1$–$\alpha 4$ discharge in synchrony but within different ranges of frequency. There is an alternation of activity between units $\beta 1$ and $\alpha 1/\alpha 2$.

is significant that units $\alpha 2$ and $\alpha 3$ also show a tendency to oscillate in this manner on cycles $\tau C 2$ and $\tau C 3$.

Unit $\alpha 2$ is active only when $\alpha 1$ is discharging, and is also recorded in nerve B4 (lower channel). This is a larger-amplitude unit than $\alpha 1$, and the discharge frequency is lower ($\frac{1}{2}$–1 p.p.s.). Although its onset is at approximately the same time as $\alpha 1$, it switches off before $\alpha 1$ on the second and third cycles. The change in interspike interval closely follows that of burst $\alpha 1$, and there is an inflexion in the curve, which is again interpreted as a tendency to oscillate.

Unit $\alpha 3$. This burst, which is recorded in nerve B2 (3rd and 4th channels), is of similar spike amplitude to unit $\alpha 2$, and also discharges at about the same frequency. The change in inter-spike interval also closely parallels that of unit $\alpha 2$, although it is possible that this unit also discharges in the τB period, since the spikes cannot be detected because of the presence of a number of other units in that period.

Unit $\alpha 4$. This is a large-amplitude burst recorded in nerve B3 (upper channel), whose inter-spike interval change also tends to follow that of units $\alpha 1$, $\alpha 2$, and $\alpha 3$, and whose discharge frequency is slightly higher than that of units $\alpha 2$ and $\alpha 3$. This burst differs from other τA-period bursts in that it also fires at low frequency during the τB period, and tends to have a sharp cut off at some point in each τA period. The point at which this burst terminates is simultaneous with the onset of burst $\alpha 5$ on the second and third cycles, and it significant that when burst $\alpha 5$ terminates at the end of
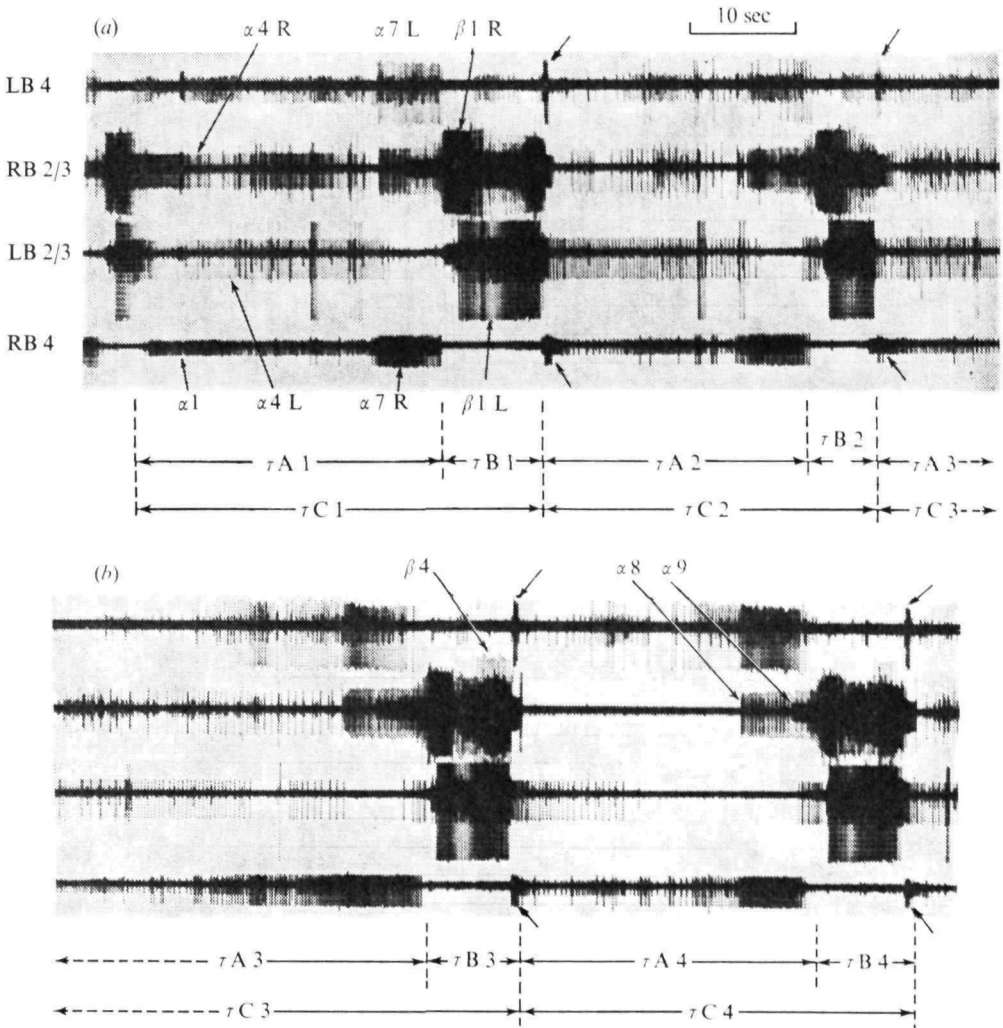
Fig. 14. Continuous 4-channel extracellular recording showing four cycles of activity (τC 1–τC 4). Units α 1, α 4, α 7, α 8, and α 9 discharge in each τA period, and β 1 and β 4 in each τB period. Bursts α 4 (L) and α 4 (R) are out of phase on the first cycle (τC 1), and unit α 4 (R) fires a short burst on other cycles. There is a tendency for unit α 4 (L) to shorten as burst α 7 lengthens on successive cycles. Arrows indicate a short burst in unit α 7 at the onset of each τA period (see also unit d in nerve RB 4, Fig. 12).

each τA period unit α 4 resumes firing and discharges at low frequency during the following τB period. This suggests that there is an alternation of activity between units α 4 and α 5.

Unit α 5. This is a small-amplitude unit recorded in nerve B2 (channel 4). The discharge frequency is high (2–3 p.p.s. maximum), and nearly constant. The burst begins at the termination of unit α 4, and is of relatively short duration, terminating at the onset of the following τB period. There is a possibility that this unit also fires during the following τB period since, like α 3, it cannot be detected in the τB period because of the presence of other units on this channel of the recording.

Unit α 6. This is a poorly defined burst, which has been included for completeness,

is of large amplitude and there are only about 20 spikes/burst. The first spikes occur slightly before the onset of burst $\alpha 5$, and simultaneous with the termination of burst $\alpha 4$.

(ii) τB-period bursts

Three bursts have been identified in the τB period, which have been labelled as $\beta 1$, $\beta 2$ and $\beta 3$ in Figs. 12 and 13.

Unit $\beta 1$. This is of larger amplitude and discharges at a higher frequency (1–10 p.p.s.) than any other burst in the sequence. It is recorded in nerve B2 (3rd and 4th channels) and discharges for the whole of the τB period on each cycle, alternating exactly with burst $\alpha 1$. The frequency is highest at the onset of the burst and gradually declines. But the inter-spike interval change plotted on a logarithmic scale shows that the change in frequency cannot be fitted by a simple exponential process. Both the burst duration and the curvature of the line which has been fitted to the points decline on successive cycles. Unit $\beta 1$ is probably unit 5 of Fig. 10 and unit *e* of Fig. 2. The presence of two units (4 and 5) of comparable amplitude, frequency and timing in Fig. 10 suggests that there may also be two units on the third channel of Fig. 12 whose amplitude cannot be distinguished because of the overlap of channels 3 and 4.

Unit $\beta 2$. This is a short burst of 16–20 small-amplitude spikes, which occurs in nerve B3 (upper channel). On the first cycle it begins $\frac{1}{2}$ sec. after the high-frequency peak of burst $\beta 1$, but on the 2nd and 3rd cycles it begins before burst $\beta 1$ reaches peak frequency. The inter-spike interval change shows a gradual rise to a maximum of < 2 p.p.s. followed by a gradual fall on each cycle.

Unit $\beta 3$. This is a large amplitude unit also recorded on nerve B3 (upper channel). It discharges for most of the τB period on each cycle, and the frequency change following a gradual rise and fall in the range of $\frac{1}{2}$–1 p.p.s. The onset of the burst occurs approximately at the point of unit $\beta 2$, and unit $\beta 3$ itself does not terminate until the end of the τB period, so that units $\beta 2$ and $\beta 3$ span the τB period.

The main features of the sequence as described so far will now be summarized:

1. There is an exact alternation of units $\alpha 1$ and $\beta 1$, which define the durations of the τA and τB periods respectively.

2. The inter-spike interval changes of units $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 4$ parallel one another closely on all cycles, although unit $\alpha 4$ terminates before the other units, and also discharges during the τB period.

3. There is evidence of an alternation of activity between units $\alpha 4$ and $\alpha 5$ on the second and third cycles.

4. Units $\beta 2$ and $\beta 3$ together span the τB period, and only partially overlap with each other.

A more complex sequence

Fig. 14 is a 4-channel extracellular recording from nerves B4 (left and right) and B2/3 (left and right). Four cycles of sequential activity are shown (although about twenty cycles were recorded) since this particular sequence showed some stability. The inter-spike interval changes for units identified as $\alpha 1$, $\alpha 4$, $\alpha 7$, and $\beta 1$ have been plotted in Fig. 15 for four cycles. These bursts have been grouped into two pairs in Fig. 15 ($\alpha 1/\beta 1$, upper part; and $\alpha 4/\alpha 7$, lower part). The purpose of this grouping is to
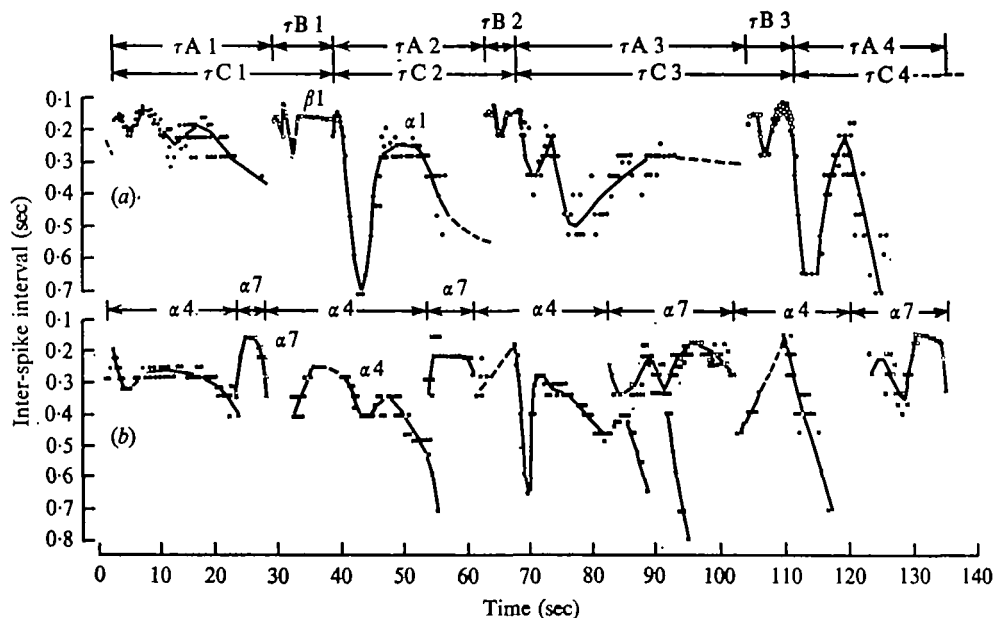
Fig. 15. Inter-spike interval changes of four units in the sequence shown in Fig. 14. (a) Oscil-
lation 1, between α 1 and β 1. (b) Oscillation 2, between α 4 and α 7. There is evidence of alter-
nation of activity between the two units in each case. Note the tendency for frequency to fall in
the middle of each burst.

suggest that this sequence may be interpreted as two two-burst oscillations which are
displaced relative to each other. These oscillations will now be considered in turn.

Oscillation 1 (α 1–β 1)

This is the oscillation described in the previous sequence and analysed theoretically
(R. M. Rose, in preparation). It is shown in the upper part of Fig. 15. Bursts α 1 and
β 1 can be identified by the usual criteria. Burst α 1 is recorded in nerve B4, is of small
amplitude, and discharges at quite a high frequency. Burst β 1, which is of large
amplitude, discharges at a higher frequency than any other burst and is recorded in
nerve B2/3. It was shown previously that the inter-spike interval change of unit β 1 is
almost exponential. The unusual feature about the α 1–β 1 alternation of Fig. 16 is
that both units show a fall in frequency in the middle of the burst. A similar effect has
been shown previously for unit α 1, but in Fig. 15 the fall in frequency in the middle of
the burst is more exaggerated than in Fig. 14 even if it is taken into account that a
linear scale is being used in Fig. 15 compared with a logarithmic scale in Fig. 13.
Unit β 1 shows a distinct fall in frequency in the middle of the burst compared with
the usual near-exponential decay. Furthermore, the spike repetition frequency of
unit α 1 falls on successive cycles, there being fewer spikes/burst in later cycles. Unit
α 1 (R) also begins firing before α 1 (L) and sometimes terminates earlier.

Oscillation 2 (α 4–α 7)

It has been pointed out previously that unit α 4 terminates before the τA period is
completed on each cycle. In this particular sequence a unit which has been labelled as
α 7 begins to fire when unit α 4 cuts off, and continues until nearly the end of the τA
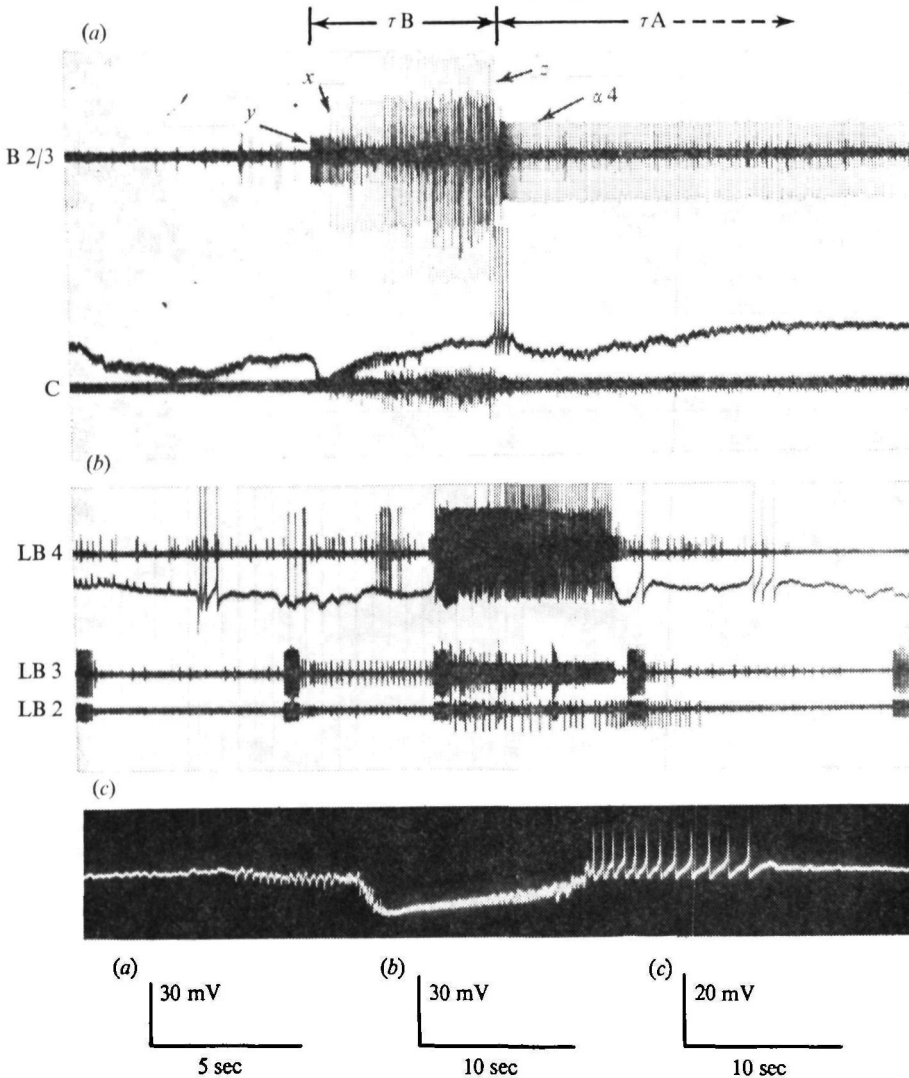
Fig. 16. (a) Simultaneous recording from nerves B2/3 and C, extracellularly, and an un-identified cell intracellularly. A hyperpolarizing waveform (I.L.D.) occurs during the period when bursts x, y and z are discharging, which is followed by a short burst due to rebound excitation.

(b) Simultaneous recording from three buccal nerves, extracellularly, and another unidenti-fied cell intracellularly. This cell is excited as a group of bursts are recorded in the buccal nerves.

(c) Intracellular recording to show more detail of I.L.D. and rebound excitation in another unidentified cell.

period, when it suddenly cuts off, and unit $\alpha 4$ begins to fire again. As the duration of the $\alpha 4$ burst gets shorter on successive cycles, burst $\alpha 7$ becomes longer, suggesting an alternation of activity between the two units. There are several interesting features in this interaction. Unit $\alpha 4$ can be identified by the usual criteria – the tendency to oscillate (R. M. Rose, in preparation) and its presence in nerve B2/3. However, there are two main differences in the behaviour of this unit compared with previous

recordings. First, burst $\alpha 4$ (L) and $\alpha 4$ (R) are not in phase on the first cycle, and
unit $\alpha 4$ (R) only fires a short burst on the other cycles. On cycle $\tau C 1$, burst $\alpha 4$ (R)
discharges at a higher frequency than $\alpha 4$ (L), and also the onset and termination of
burst $\alpha 4$ (L) decreases on successive cycles, and on the fourth cycle there is a very
rapid fall in inter-spike interval change. This recording therefore suggests that if a
burst in one ganglion shortens, the partner burst is gradually shortened and brought
into phase.

The other burst involved in this alternation has been labelled as $\alpha 7$, although it is
possible that it is unit $\alpha 2$ of previous recordings, since it is of similar amplitude to
unit $\alpha 2$ and has its axon branch in nerve $B 4$. The main difference between $\alpha 2$ and $\alpha 7$
is in the firing pattern, since in Fig. 12 the inter-spike interval change for unit $\alpha 2$
paralleled that of units $\alpha 1/\alpha 3/$ and $\alpha 4$, whereas unit $\alpha 7$ in Fig. 15 alternates with
unit $\alpha 4$.

Three other bursts, $\beta 4$, $\alpha 8$, and $\alpha 9$, have been labelled in Fig. 15. Unit $\beta 4$ is
probably the same burst as g (and h), labelled in Fig. 11, since its onset is late in the
τB period and the amplitude and frequency are similar. Burst $\alpha 8$, which occurs in
nerve $B 2/3$, begins firing 4–5 sec. before the end of the τA period in each cycle.
Simultaneous with the onset of this burst there is a sudden rise in frequency of units
$\alpha 7$ (R) and $\alpha 7$ (L) on each cycle. Another small-amplitude burst is also recorded in
nerve $B 2/3$ and has been labelled as $\alpha 9$. This burst also occurred in the recording of a
decaying oscillation shown in the theoretical paper on these sequences (R. M. Rose,
in preparation).

Intracellular recording

Fig. 16a is a recording made from an unidentified cell in lobe $L 1$ during a single
feeding cycle. The activity of nerves $B 2/3$ (upper channel) and C (lower channel) is
also shown. The τA and τB periods have been identified on the basis of the charac-
teristic frequency change of one unit, which can be identified as $\alpha 4$. The three other
units in the τB period have been labelled as x, y, and z, since it is not possible to relate
these to identified units with certainty. It is probable, however, that unit z is burst $\beta 4$.
A large-amplitude hyperpolarizing waveform occurs in the unidentified cell, which is
simultaneous with bursts in the τB period. Immediately following the τB period this
cell rebounds from the inhibition to produce a short burst of action potentials. Simul-
taneous with this burst, unit $\alpha 4$ begins to fire at high frequency, suggesting rebound
excitation in this cell also. The change in inter-spike interval of unit $\alpha 4$ again shows
evidence of an oscillation, although this has not been plotted in this case. Fig. 16c is an
intracellular recording from another unidentified cell in lobe 1 which also shows
inhibition of long duration (I.L.D.) and rebound excitation. This is shown simply as
another illustration of this effect in the buccal ganglion. In Fig. 16b an example of an
excitatory connexion is given. A high-frequency burst occurs in an unidentified cell
which is simultaneous with bursts in three buccal nerves. These recordings illustrate
the problem of attenuation of post-synaptic potentials, which may lead to difficulties
in further investigations of specific connexions between identified cells.

DISCUSSION

This paper has provided evidence of an alternation of activity between bursts in the τA and τB periods. In another paper (R. M. Rose, in preparation) this alternating activity has been described mathematically in terms of van der Pol's equation (van der Pol, 1926) for a relaxation oscillation. In this theoretical paper it has been suggested that alternation of burst activity may result from mutual inhibition between cells in the τA and τB periods. Preliminary intracellular recordings showing I.L.D. waves during the τB period support this theory. Although this paper provides additional details of bursts occurring in the sequences, the main contribution to the theoretical ideas is the finding that units α1, α2, α3, and α4 discharge synchronously but within different ranges of frequency. The basis of the theory is that the sequences could be interpreted as an oscillation between two neurones. Solutions of van der Pol's equation are single waveforms but it now seems that the idea of a simple oscillation between two neurones could be extended to an oscillation between two groups of neurones, and these groups represented as a set of waveforms of different amplitudes. The oscillation is thus split into 2–4 levels, with one neurone contributing to each level. In the τA period, the inter-spike interval changes of units α1, α2, α3, and α4 parallel each other, but each unit discharges within a different range of frequency. Similarly, evidence has been given (Fig. 11) of synchronous bursts in the τB period, each discharging within a different range of frequency. In the buccal ganglion of *Archidoris* (Rose, 1971 b) the inter-spike interval changes of different bursts have also been shown to parallel each other within different frequency ranges. This synchrony within each group could result from electrical connexions or common input to the cells. Both of these possibilities have been shown to exist other buccal in ganglia. Levitan *et al.* (1969) have described electrical connexions between 5 neurones in each buccal ganglion of *Navanax*, and Gardner (1969) has shown that there are two interneurones in each buccal ganglion of *Aplysia californica*, each with inputs to six follower cells. There is little point in speculating on which of these possibilities exists in the buccal ganglion of *A. depilans*, since a detailed intracellular study would provide the necessary information.

One important theoretical question is whether the ideas worked out for a single oscillator can be generalized to interacting oscillators. An attempt has been made in this paper to interpret a more complex sequence as two interacting oscillations. It is suggested that in dealing with interacting oscillators the main problem is to define the type of interaction. Once this has been done it would be possible to generalize the results to a larger group by solving a set of simultaneous differential equations. Previous attempts at this kind of analysis (Pavlidis, 1969) have considered a simple form of interaction such as reduction of the frequency of one oscillator by another as being the principle of interaction. One of the problems in future work on the buccal ganglion of *A. depilans* is to attempt to identify more than one oscillator, and to define interactions between two oscillators quantitatively.

SUMMARY

1. The activity of the buccal ganglion of *Aplysia depilans* is manifested as regular and sequential bursts of nerve impulses.

2. Regularly firing bursts are seen in the absence of feeding movements.

3. Sequences of bursts lasting for several minutes have been recorded during feeding movements induced by a natural stimulus.

4. A feed-back system has been used to produce bursts in certain other cells synchronous with the regularly firing units.

5. During sequences of bursts there is an alternation of activity between the two groups of neurones.

6. One group is made up of four cells discharging synchronously but within different ranges of frequency.

7. A mathematical model of this activity will be presented in another paper.

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