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A METHOD FOR RECORDING ELECTRICAL ACTIVITY FROM THE BODY WALL NERVE NETS IN SEA ANEMONES

KWANGWOOK CHO AND IAN D. MCFARLANE
Department of Applied Biology, University of Hull, Hull HU6 7RX, UK

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Summary

Glass microelectrodes were used to record electrical activity from thin rings cut from the column of the sea anemone *Calliactis parasitica*. This is the first time that pulses have been recorded from the nervous system in the column. Three pulse types were detected, types A, B and C. Type A pulses are probably associated with neurones of the through-conducting nerve net. Type B pulses may be from the endodermal slow conduction system (SS2). Type C pulses have not previously been recorded and are thought to represent activity in a local nerve net. At this stage we cannot positively state whether the recordings are intracellular from endodermal myoepithelial cells or are extracellular from the sub-epithelial region.

Key words: electrophysiology, nerve net, sea anemone, Cnidaria, *Calliactis parasitica*.

Introduction

Much of what we know about the behavioural physiology of sea anemones comes from studies using extracellular suction electrodes attached to tentacles. Results show that anemones have multiple conduction systems (McFarlane, 1973*a*): a through-conducting nerve net (TCNN) and two slow systems, SS1 and SS2. These are probably three separate, but interacting, nerve nets. The SS1 is ectodermal and a single, evoked SS1 pulse spreads over the entire ectoderm. In *Calliactis parasitica*, the SS1 responds to food and also to shell contact during shell-climbing behaviour (McFarlane, 1976). In *Stomphia coccinea*, SS1 sensory responses coordinate escape swimming (Lawn, 1976). SS1 activity causes pedal disk detachment and relaxation of ectodermal muscles (McFarlane, 1969). The SS2, in contrast, is restricted to the endoderm. A single, evoked SS2 pulse spreads throughout the whole endoderm (McFarlane, 1969). The SS2 and the TCNN act together to coordinate the spontaneous contraction cycle (McFarlane, 1974*a*). SS2 activity inhibits contractions of body wall circular and parietal muscles (McFarlane, 1974*b*). Spontaneous SS2 pulses may arise from a sensory system detecting stress, perhaps between antagonistic body wall muscles (McFarlane, 1974*a*).

The TCNN was first described by Pantin (1935). TCNN neurones occur in all regions except the body wall ectoderm. TCNN pulses, normally evoked by touch, spread without restriction and excite muscle groups to give either slow or fast contractions (Batham and Pantin, 1954; Ross, 1957). TCNN bursts can also arise from pacemaker neurones (McFarlane, 1973*b*). Other components of the nervous system have been proposed; for example, a local net to coordinate local contraction (Pantin, 1935). Nervous activity has a restricted spread in a local nerve net.

Suction electrodes suffer the limitation that electrical activity can normally only be recorded from tentacles, although TCNN activity has been detected in mesenteries (Robson and Josephson, 1969). This means that electrical activity restricted to the body wall (i.e. local) will not be recorded by electrodes on tentacles. The body wall is, however, an important region: Grimmelikhuijzen *et al.* (1991) report numerous nerve cells at the bases of mesenteries. Voltage-clamp experiments have been performed with isolated body wall myoepithelial cells (Holman and Anderson, 1991) but microelectrodes have not previously been used to record nervous activity.

Here, we introduce a technique to record electrical activity from the nerve nets in the body wall of sea anemones. We believe this will provide valuable insights into the primitive nervous system.

Materials and methods

Calliactis parasitica were supplied by the Marine Station, Roscoff, France, and were kept at 14 °C. Before dissection, they were kept at 8–10 °C for at least 30 min; this reduces their activity and makes them easier to dissect. Anaesthetics were not used as they delay recovery and cause abnormal responses. After drying with soft tissue paper, the upper quarter of the animal was cut off and horizontal slices, 2–3 mm thick, were taken from the remainder. Each slice was a whole body wall ring. The main problem with these preparations is the presence of excessive amount of debris and mucus: the following

procedures were designed to remove these contaminants. Column slices were washed with artificial sea water (ASW; in mmol l^{-1}: NaCl 395; KCl 10; $CaCl_2$ 10; $MgCl_2$ 50; Hepes buffer 10; pH 8.0; Holman and Anderson, 1991) at 5 °C. A selected slice was agitated for 5 min in ASW and then transferred to fresh ASW; this removes some mucus and loose debris. The preparation was then treated with a primary digestive solution for 30 min. We used two kinds of solutions for digestion. The first consisted of 3.75 mg ml^{-1} papain (Sigma Type IV) in ASW, activated with 0.5 mg ml^{-1} dithiothreitol (Sigma) at pH 8.0 and 10 °C (Holman and Anderson, 1991). The slices were then treated with the second digestive solution for 30–50 min. This consisted of the first digestive solution, plus 1.5 mg ml^{-1} Protease (Sigma type XIV) in ASW at pH 8.0, activated with 1 mg ml^{-1} dithiothreitol at 12–15 °C. The tissue was gently shaken at 3–5 min intervals and, after digestion, was washed with fresh ASW at 10 °C.

The column slice was pinned in a specially designed chamber stage measuring 8 cm×8 cm, with a well, 4 cm in diameter and 0.5 cm deep, cut in the centre. An inlet tube connected to a header tank allowed the ASW to be changed when required. Glass microelectrodes (60–70 MΩ tip resistance, tip diameter 1 μm or less) were filled with 3 mol l^{-1} KCl. The stimulating microelectrode was a silver wire sealed in a glass capillary. The amplifier was a Nihonkoden MEZ-8101 and recordings were stored on computer using a Mac Lab 2 with Mac Chart and Scope software.

The recording electrode was attached to a Narishige micromanipulator with the head set at 60–90 ° to the main chamber stage. The chosen recording site was the endoderm close to where the mesenteries insert onto the body wall. It was important to maintain the preparation and the recording electrode at roughly the same temperature. Stimuli (10 V, 0.2 ms) were passed through the recording electrode: contact with the endodermal cell layer was shown by the appearance of a prolonged depolarization after the stimulus artefact. Penetration of the endodermal cell layer was marked by a membrane potential drop to around −60 mV. In most preparations, this potential was held for approximately 5 min, but it then slowly drifted to around −20 to −30 mV. Continuous recordings lasted up to 25 min. During recording, the ASW was changed once to reduce contamination by excess mucus. The stimulating electrode was placed in the endoderm, approximately 1 cm from the recording site.

Results

Three different pulse types (types A, B and C) could be recorded after electrical stimulation of column slices (Fig. 1). At any given recording site, we observed one of three different responses: type A and type B together, or type B alone, or type C alone. As Table 1 shows, types A and B together was the most frequent response. So far, no recordings have shown type A alone.

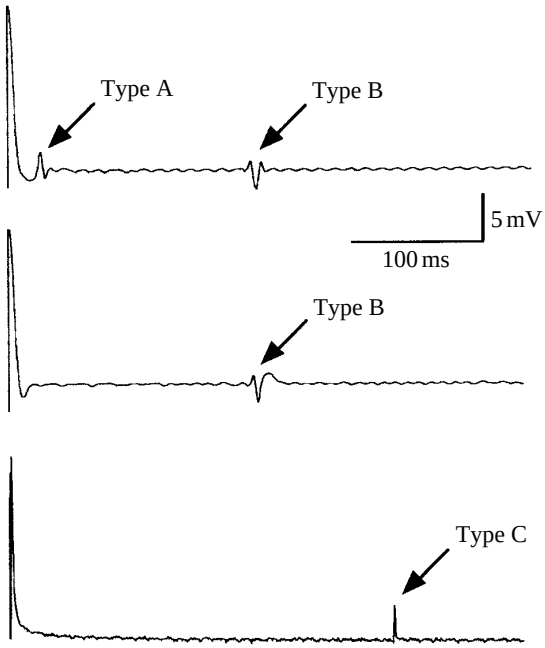

Fig. 1. Three separate recordings from the body wall endoderm of *Calliactis parasitica.* Each shows the response to a single shock (large stimulus artefact at start of trace). Three different types of pulses could be recorded. At some sites, types A and B were seen together, other sites showed only type B or type C.

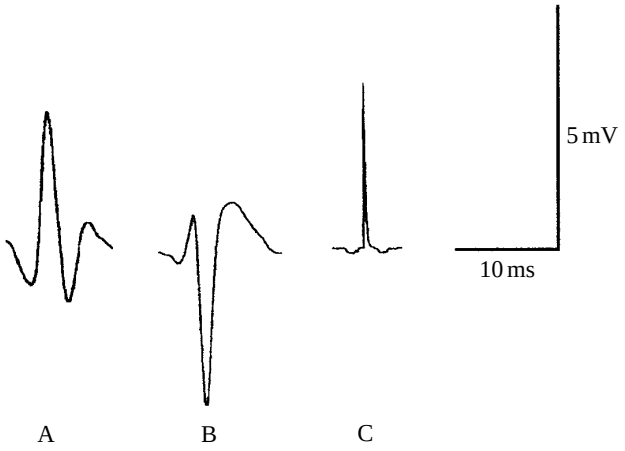

Fig. 2. Examples of the individual pulse types shown at high gain.

Table 1. *The frequency of occurrence of combinations of pulse types A, B and C in recordings from column slices of* Calliactis parasitica

	Number of examples	Occurrence (%)
Types A+B together	28	45.9
Type B alone	12	19.7
Type C alone	21	34.4

Each pulse type had a distinctive shape (shown at high gain in Fig. 2). Type A was approximately 10 ms in duration and

Table 2. *The specificity of the three responses recorded from column slices of* Calliactis parasitica

	Refractory period (ms)	Conduction velocity ($cm\,s^{-1}$)
Type A	12.0±2.0	44.4±0.4
Type B	39.1±2.1	8.4±0.8
Type C	15.4±1.9	5.1±0.5

The values indicate the mean and standard deviation (N=6).

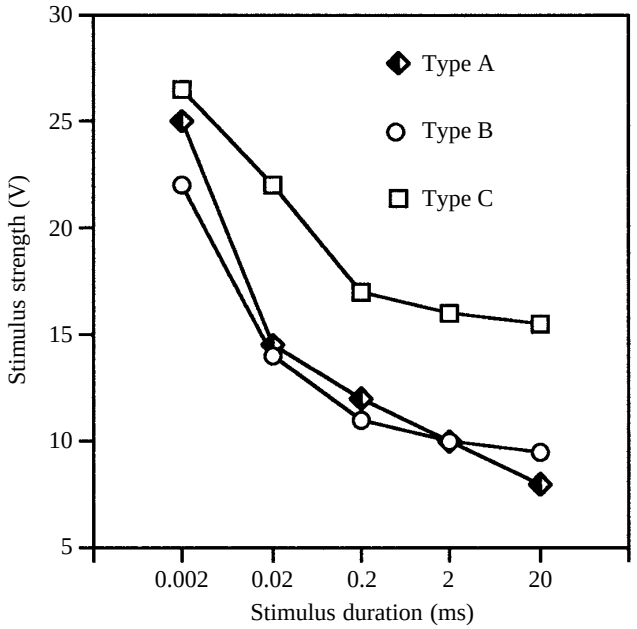

Fig. 3. Strength/duration curves for the three pulse types recorded from column slices of *Calliactis parasitica*. The readings recorded for each duration are an average of five trials. For purposes of clarity, error bars are omitted: maximum standard deviation is ±9 %.

had an amplitude of 3 mV. This pulse was bi- or triphasic and was mainly positive. Type B lasted 10–25 ms and had an amplitude of 3 mV. Again, it was generally triphasic, but, in this case, was mainly negative. Type C was distinctively different in usually being a brief (much less than 10 ms) positive monophasic event. This pulse was up to 4 mV in amplitude. The three pulse types had different relative refractory periods (Table 2), that of type B being significantly longer than that of types A and C. There were also significant differences in conduction velocity between the three pulses (Table 2). A comparison of the strength/duration curves (Fig. 3) also shows differences between the pulse types. Type C had a higher threshold than the other two pulses. Type B had a slightly lower threshold than type A, except at longer stimulus durations.

Types A and B were only recorded in response to electrical stimulation. Type C, however, could be evoked by electrical stimulation but also appeared spontaneously, with a normal mean frequency of 1.5±0.5 pulses min^{-1}.

Discussion

We have recorded three different pulse types from column slice preparations; as the pulses have distinct properties, they must represent activity associated with three separate conduction systems. Before discussing what these systems are, we must establish the recording site. The electrode cannot have penetrated individual nerve cells because, although nerves run in the extracellular spaces between the myoepithelial cells (Robson, 1957), actinian nerve cell bodies are generally less than 5 μm in diameter (Batham *et al.* 1960). In our experiments, every attempt resulted in successful recordings, showing that the target is a continuous layer. The observation of a −60 mV potential suggests that the recordings were intracellular, the most likely recording site being the endodermal myoepithelial cells. The recorded events would then be either synaptic potentials, resulting from innervation, or propagated responses in the membrane of the myoepithelial cells.

However, the small size of the pulses argues against them being propagated events, and the waveforms are more complex than might be expected for synaptic potentials. The simplest explanation is that the recordings are extracellular and that the observed membrane potential is actually a transepithelial potential. Unfortunately, we cannot definitely identify the recording site because the high resistance of our recording electrodes has thwarted attempts to label by ionophoretic injection of Lucifer Yellow. Until further evidence is available, we will assume that the recordings are extracellular from units in the column nervous system.

Another matter for discussion is the identity of each pulse type. Type A has a low threshold and high conduction velocity: the values show that type A corresponds to the TCNN pulse seen in suction electrode recordings (Josephson, 1966). In whole animals and some isolated preparations, the TCNN is spontaneously active (McFarlane, 1973*a*). We have not seen spontaneous type A pulses, possibly because the integrity of the hydrostatic skeleton has been lost and there is no load on these column slice preparations.

Apart from TCNN pulses, column slices should show SS2 activity: it seems reasonable therefore to assume that either type B or type C corresponds to an SS2 pulse. Both types B and C have a conduction velocity considerably lower than that of type A and within the range of values recorded for the SS2 (McFarlane, 1969). Like the TCNN, the SS2 is spontaneously active in whole animals and some preparations (McFarlane, 1973*a*), but is inactive in preparations where the integrity of the hydrostatic skeleton is lost (I. D. McFarlane, unpublished observation). Type C pulses do, however, appear spontaneously. The only evidence that type C, rather than type B, represents the SS2 is that in suction electrode studies (McFarlane, 1969) the SS2 has a high stimulus threshold. In those studies, however, stimuli were applied through a suction electrode attached to the outside of the column, whereas in the present study stimuli were given through a microelectrode placed directly on the endoderm.

Pulse type C differs from the other pulses in being

spontaneously active. The third conducting system seen with extracellular suction electrodes on tentacles, the SS1, is ectodermal and, therefore, no associated pulse should be seen in endodermal recordings from column slices. In addition, the SS1 is not spontaneously active. If type C pulses do not correspond to pulses seen in tentacles, the implication is that pulses in this system do not spread everywhere throughout the body; in other words, it is a local nerve net. It is known that endodermal muscles can contract independently of input from the TCNN (McFarlane, 1974*a*) and, therefore, this system may provide the pathway for such excitation.

There is one serious problem with the interpretation of our results. If the recordings are extracellular and are detecting single units, how do we account for the observed pairing of type A and type B pulses? One possibility is that this particular unit shows two action potentials. Neurones with both Na^+ and Ca^{2+} spikes have been described in the jellyfish *Aglantha digitale* (Mackie and Meech, 1985).

The advantages of this technique over extracellular recordings with suction electrodes are (a) that electrical activity in the column can be monitored directly, (b) that an additional conduction system (shown by pulse type C) can be detected and (c) that the action of neuropeptides on the conducting systems can be easily investigated. Future work will aim to confirm the identities of these pulses and to study how the actinian neuropeptides isolated by Grimmelikhuijzen and co-workers act on the column nerve nets.

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