## Stimulation and Recording from Single Cells in Whole Animal Preparations of *Aplysia*

Knowledge of invertebrate central nervous systems has been advanced a great deal in the past 15 years by the use of *Aplysia* ganglion cells in investigations of the basic mechanisms of synaptic transmission[1,2], and the electrophysiological anatomy of individual neurones[3,4]. More recently the isolated ganglion preparations have been used to study modifications in responses which suggest analogies with behaviour of higher animals and especially to different types of learning[5,6]. However, little has been done to correlate responses of the central nervous system of whole animal preparations.

In such preparations HUGHES and TAUC[7] studied the effect of mechanical stimulation of the periphery on the responses of single neurones of the abdominal ganglia and especially the so-called giant cell (RGC). Few investigators have reported work on the effect of stimulating single neurones on the overt activity of this animal. Stimulation of the RGC did not seem to evoke any overt response[8]. One reason for the lack of responsiveness of whole animal preparations is due to their deterioration following the loss of haemolymph which constitutes approximately 40–60% of the body weight[9]. The important function of this fluid as a hydrostatic skeleton inevitably leads to a reduction in the ability of the animal to make co-ordinated movements because of the general loss in body tone.

In recent experiments, a technique has been developed for the suspension of *Aplysia fasciata* and, following the opening up of the body cavity, the loss of fluid from the tissue spaces has been reduced by clamping. Detailed studies have been made of the activity and responses to stimulation of cells in the left pleural and pedal ganglia. These ganglia are expected[7] to be more closely involved in the normal movement patterns of these animals than the abdominal ganglia.

It is of great interest that quite definite and reproducible, though localized contractions may follow the direct stimulation of certain neurones in the pedal ganglia (Figure 1). These localized responses occurred on the ipsilateral side, and were correlated with the path of an axon of the stimulated neurone in the nerve of the responding region. Together with the invariable activation of these cells during spontaneous or evoked movements of such regions (Figure 1), the evidence strongly suggests that these are motor neurones. Stimulation of left pleural ganglion cells gave rise to more complex effects which were often bilateral in nature. There was generally a long latency before movements resulting from stimulation of the pleural ganglion cells became maximal. Furthermore, the effects were not constant and they tended to decline more or less rapidly with successive presentations of similar stimuli. Several sorts of movement were recorded depending on the type of cell stimulated.


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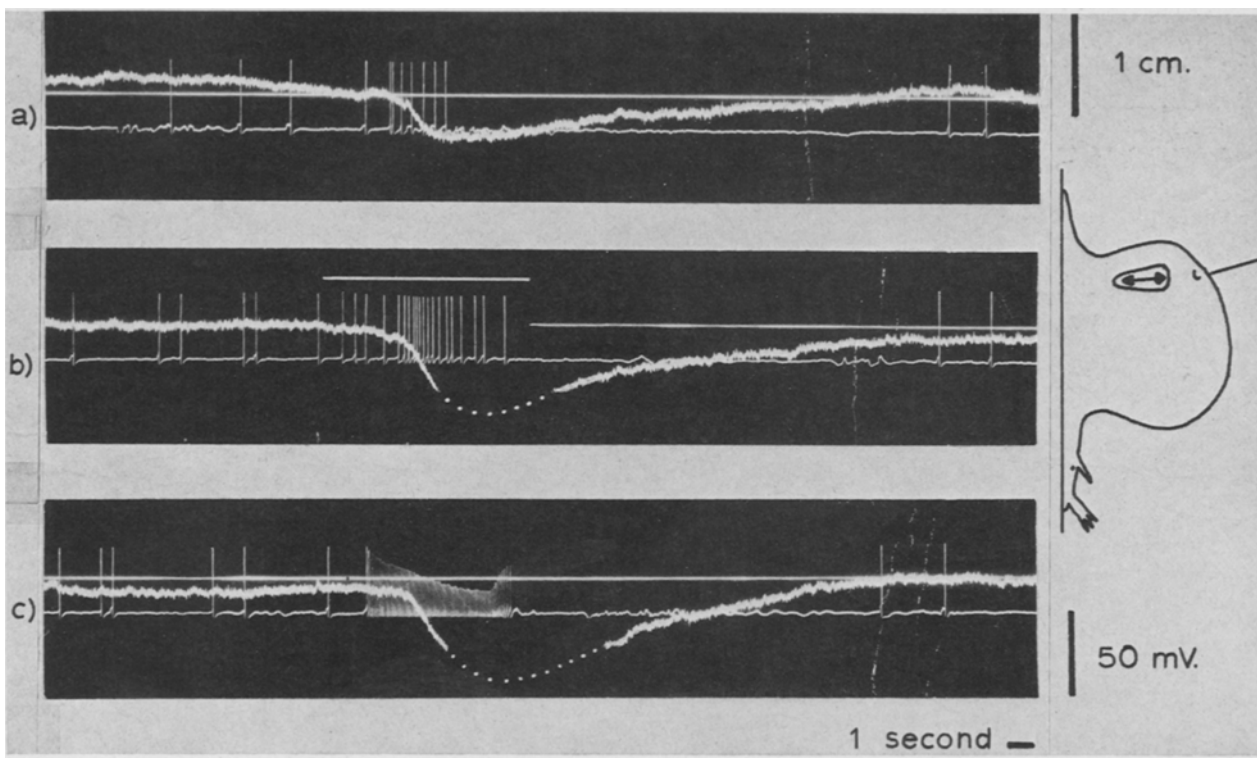


Fig. 1. Pedal motor neurone. Transducer attached to the ipsilateral parapodium as shown in the inset diagram, on which the part of the parapodium contracting is also indicated. (a) Spontaneous levation movement of the parapodium. (b) Parapodial levation evoked by light tactile stimulation of the tip of the siphon. (c) Levator muscles in the motor field of this neurone activated by depolarizing current applied to the motor neurone through the stimulating electrode.

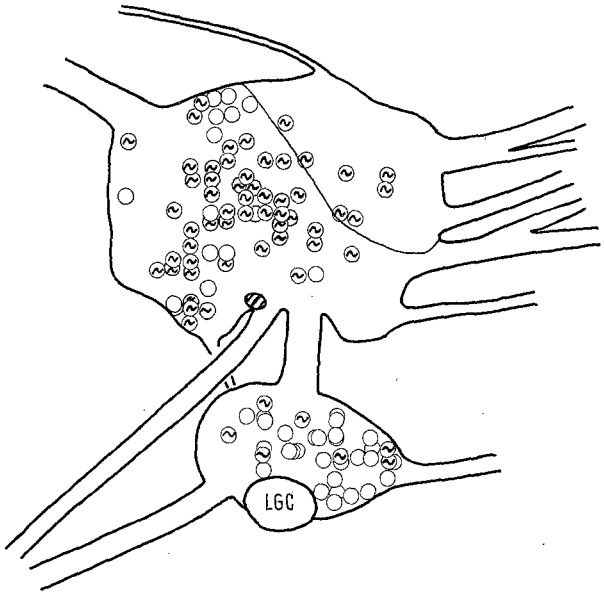


Fig. 2. Distribution of 'spontaneous' spike activity recorded intrasomatically from *Aplysia* left pedal and pleural ganglia. Each circle indicates a penetration localized by means of a grid superimposed over the ganglion outline.

swimming does not appear to be an escape response in *Aplysia*.

Intracellular recordings from pleural and pedal ganglion cells revealed comparable differences in their resting and synaptically evoked activity; cells in the pedal ganglion were generally characterized by the presence of a resting discharge which was absent from the majority of pleural cells (Figure 2). Studies of the types of responses evoked by tactile stimulation has shown a wide variety of effects. Some cells respond to more localized stimuli of a single modality, whereas others respond to stimulation of a wider receptive field. The former type have not been found in the pleural ganglion whereas the latter are common there (Figure 3). From both the direct stimulation and recording experiments it is concluded (a) that the pedal ganglion may be regarded as a lower centre containing motor neurones and possibly low-order interneurones, and (b) that neurones in the pleural ganglia are involved in rather more complex behavioural responses. If this interpretation is correct, the precise function of the much branched peripheral axons of the LGC and other pleural neurones remains quite perplexing[11].

*Résumé.* Nous avons introduit des microélectrodes dans les neurones des ganglions pédieux et pleural de l'Aplysie suspendue d'une manière qui permettait des mouvements

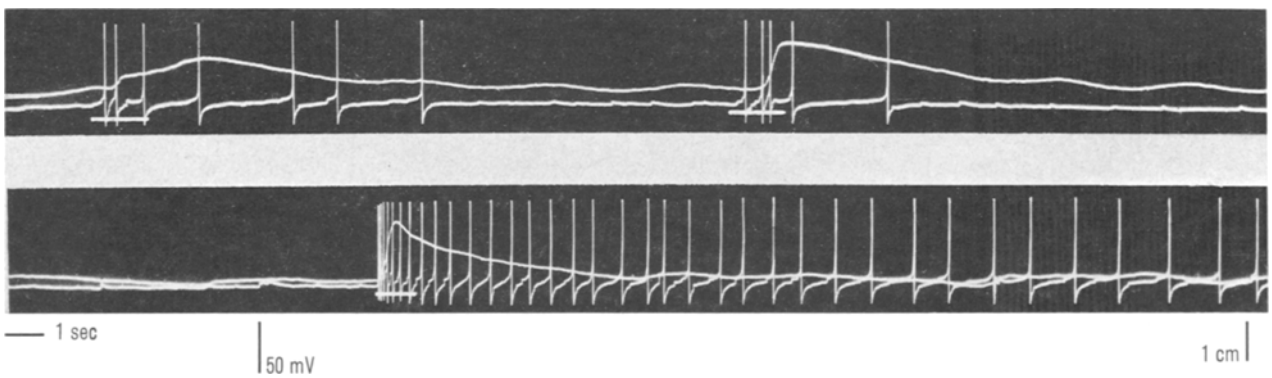


Fig. 3. Pleural neurone responding with excitation to (in order) touching a paintbrush on the water surface, touching the contralateral tentacle and touching the siphon. The transducer records upward movement of the ipsilateral parapodium.

Of special interest was the effect of direct intracellular stimulation of the left giant cell (LGC). The suspended preparation often showed up and down movements of the parapodia and, following stimulation of the LGC, these movements were accentuated particularly during the depression phase. Continuation of the stimulus during 10 sec usually resulted in a substantial total depression of the contralateral parapodium which became maximal after 20 sec. The ipsilateral parapodium showed a similar response. An additional effect of stimulating the LGC was a gradual build-up of an extension of the 'tail', apparently resulting from excitation of the circular and inhibition of the longitudinal muscles. Either one or both of these types of response was recorded in all the preparations in which the LGC was studied. Both types of response occur at the start of swimming in *Aplysia*.

It had been hoped that rather more dramatic responses would have been obtained by stimulation of this very large neurone and it is believed that this is still due to the less ideal state of the animal as compared with similar preparations of *Tritonia*[10]. Nevertheless, sufficient has been found to show many points of similarity between *Aplysia* and *Tritonia* with the important difference that

limités. Les stimulations physiologiques agissant sur des neurones pédieux sont plus simples que celles qui excitent les neurones pleuraux. La stimulation intracellulaire montre que certains neurones pédieux opèrent comme des «motor-neurones», tandis que les neurones pleuraux (la cellule géante de gauche (LGC) comprise) présentent un comportement complexe.

G. M. HUGHES, R. DE G. WEEVERS and R. W. HARTLEY

*Department of Zoology, The University, Bristol BS8 1UG (England), and Institut de Biologie Marine, Arcachon (France), 14 February 1969.*

[10] A. O. D. WILLOWS, *Physiological and Biochemical Aspects of Nervous Integration* (Ed. D. CARLSON, Symposium of the Society of General Physiologists 1968), p. 217.

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