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THE TIME COURSE OF PACEMAKER INHIBITION IN THE HYDROID *TUBULARIA*

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INTRODUCTION

Large electrical potentials, up to tens of millivolts, can be recorded with extracellular electrodes from hydrozoan coelenterates. Some of these potentials have been shown to originate from epithelial conduction (Mackie, 1965; Mackie & Passano, 1968), others may be events triggered in epithelia by underlying, possibly neuronal activity (Ball, 1971). In addition, smaller, often compound potentials are sometimes seen which may be entirely neuronal in origin. It has been much easier to record electrical events in hydrozoans than to identify their origins, and in general little is known about the specific roles of nervous systems and excitable epithelia in controlling behaviour. But the ease with which electrical events can be recorded from hydrozoa has made it possible to analyse the behaviour of these animals in terms of operationally definable components – conducting systems and pacemaker systems – even though the cellular substrates of these remain uncertain. For example, most of the spontaneous behaviour of the hydroid *Tubularia* can be accounted for on the basis of identified pacemaker systems and the interactions between them.

Both inhibitory and excitatory interactions are involved in the control of the behaviour of *Tubularia*. Activating the DOS – a conducting system which courses through the hydranth and stalk – inhibits spontaneous firing in the two principal pacemaker systems of the polyp, the HP system in the distal hydranth and the NP system in the neck region (Josephson & Mackie, 1965; Josephson & Uhrich, 1969). The overall pacemaker inhibition is greater the greater the frequency with which the DOS is activated (Josephson & Mackie, 1965). If the DOS is stimulated repetitively at once each 5 sec, inhibition of the NP system begins a few hundred milliseconds after each stimulus and lasts for several seconds. Other examples of behavioural inhibition are known in coelenterates (see summary in Josephson & Uhrich, 1969) but the inhibition mediated by the DOS in *Tubularia* is easily initiated and quantified and therefore seems particularly suitable for analysis.

The experiments described below were begun to better characterize the time course of NP inhibition, to determine the relation between the time course of inhibition and the frequency of DOS activity, and to compare the extent and time course of HP system inhibition with that in the NP system.

METHODS

The animals used were mature *Tubularia* collected at Woods Hole. The data presented are from two sets of experiments – one with intact polyps and the other with isolated hydranths.

The intact polyps were pinned by the stalk to the bottom of a dish of sea water cooled to 17–18 °C. Suction electrodes made from drawn plastic tubing were used for stimulating and recording. The electrodes had internal diameters at the tip of 50–100 μm. Two coils of silver wire in the bath served as indifferent electrodes for stimulating and recording. Suction electrodes were placed (1) on a gonophore or the gonophore stalk, (2) on the proboscis at the base of the distal tentacles, and (3) on the perisarc-covered stalk immediately below the hydranth. The gonophore electrode was used as a stimulating electrode to activate the DOS. It was found that stimulating here excites the DOS without affecting other known conducting systems and usually without directly activating pacemaker systems in the hydranth. The stimuli were 1 msec current pulses at approximately twice DOS threshold. The proboscis electrode was used to monitor DOS activity near the distal tentacles, where the DOS pulses are particularly large. The stalk electrode recorded potentials from the two principal pacemaker systems of the hydranth, neck pulses (= NPs) from the NP system and hydranth pulses (= HPs) from the hydranth system. In the perisarc-covered stalk just below the hydranth these two kinds of potentials can be distinguished easily for here they are of different polarity (Josephson & Mackie, 1965).

An isolated hydranth was prepared by cutting off the stalk where it joins the hydranth. The NP system is localized in the upper stalk and removing the stalk allows one to examine activity patterns in the HP system without those complications arising from HP–NP interactions (Hofman & Rushforth, 1967). The stimulating and recording conditions were similar to those with intact animals except that a glass suction electrode (Josephson, 1967) attached to the base of the polyp was used to record HPs in place of the stalk electrode.

With both intact polyps and isolated hydranths the DOS was stimulated at constant frequency for a number of 5 min periods. Each stimulation period was followed by a rest period of at least 10 min. The stimulus frequencies in the stimulation periods were, in order of presentation: 1 per 5, 1 per 10, 1 per 5, 1 per 20, 1 per 5, and 1 per 2·5 sec. A random ordering of the frequencies in the successive stimulation periods would have avoided possible difficulties introduced by systematic changes in the animal's behaviour during the course of the experiments, but animals sometimes became unresponsive during repeated stimulation at 1 per 2·5 sec so this, the highest frequency used, was always made the last of the series. Interspersing periods of stimulation at 1 per 5 sec between those of other frequencies allow checking for systematic changes in the animal's behaviour.

Often the DOS would suddenly stop firing following each stimulus. Usually increasing the stimulus strength or replacing the stimulating electrode restored DOS activation. In these cases the stimulus period was repeated after a 10 min rest and the experiment was continued. Complete runs were obtained from ten intact polyps; runs complete except for the final stimulation period, that at 1 per 2·5 sec, were

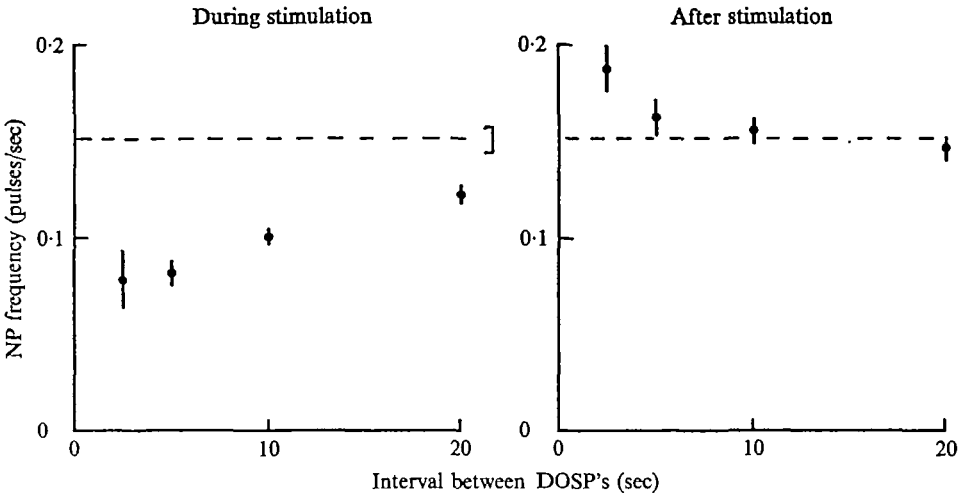

Fig. 1. NP frequency during 5-min periods with DOS stimulation and in the following 5 min periods without stimulation. This figure is based on the 10 intact animals from which complete runs were obtained (see text). Vertical bars in this and following figures indicate one standard error above and below the mean. The horizontal dashed line in this and subsequent figures indicates the expected pulse frequency based on that recorded in the 5 min periods preceding stimulation.

obtained from another two polyps. The records collected from these 12 intact polyps and 14 isolated hydranth preparations were the basis for the analysis below.

Much of the data is presented below as post-stimulus histograms. These were prepared by measuring, from pen-writer records, the interval between each NP or HP and the immediately preceding stimulus. These post-stimulus intervals were then tabulated. To facilitate comparison the histograms are expressed as average pulse frequency during post-stimulus interval bins. The average pulse frequency is the total number of pulses occurring in that interval bin divided by the total time in all included records represented by that bin. This measure, the average pulse frequency, is independent of stimulus frequency (i.e. the number of times each bin occurs in a 5 min period), the sample size (10, 12 or 14 animals) and the bin width.

The temporal resolution of the histograms is limited in part by uncertainty about pulse frequency in the bins due to statistical variability. This uncertainty is made smaller as the aggregate time represented by each interval bin in the analysed records is made longer, either by increasing the bin width, which directly limits resolution, or by increasing the length of the analysed records. To obtain sufficiently long records it has been necessary to pool records from all the animals used. The bin widths in each case have been made as narrow as seems reasonable with the available data.

RESULTS

(1) *Inhibition of the NP system*

Inhibition of NP activity during DOS stimulation is greater the higher the stimulus frequency (Fig. 1). This result, obtained by activating the DOS with gonophore stimulation, is similar to that obtained earlier from stalk stimulation (Josephson & Mackie, 1965), but the inhibition with gonophore stimulation is somewhat more pro-

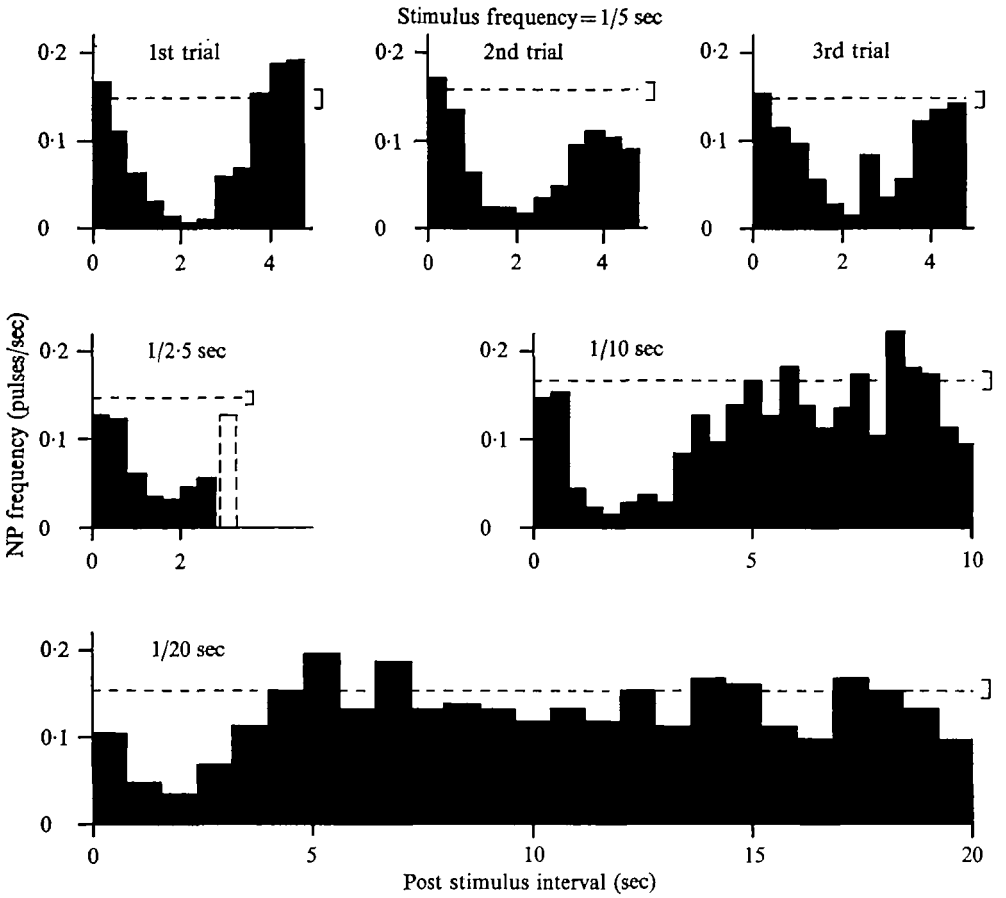

Fig. 2. The distributions of NPs in the intervals between stimuli activating the DOS.

nounced, presumably because stalk stimulation activates the DOS and a second conducting system, the TS, which can trigger NPs in the polyp (Josephson, 1965). The NP frequency in the 5 min periods immediately following stimulation was similar to that before stimulation except following stimuli at 2·5 sec intervals, in which case there was rebound from inhibition and the post-stimulatory frequency was higher then in control periods. Post-stimulus interval histograms for NPs in each of the stimulation periods are compared in Fig. 2. The histograms for the three stimulation periods with stimuli at 5 sec intervals are generally similar, indicating that there were no consistent changes in activity of the NP system during the course of the experiments. With the exception of the stimulus period with shocks at 2·5 sec intervals, the reduction of NP frequency in each histogram is similar in time course and extent. NP inhibition is most pronounced about 2 sec after a stimulus which activates the DOS, and the recovery from inhibition is complete in 4–5 sec. The time course of inhibition is most clearly seen in Fig. 3, in which data from all the stimulation periods with 1 per 5 sec have been pooled for maximum temporal resolution.

The period of NP inhibition is somewhat shorter in the histogram with 1 per 2·5 sec

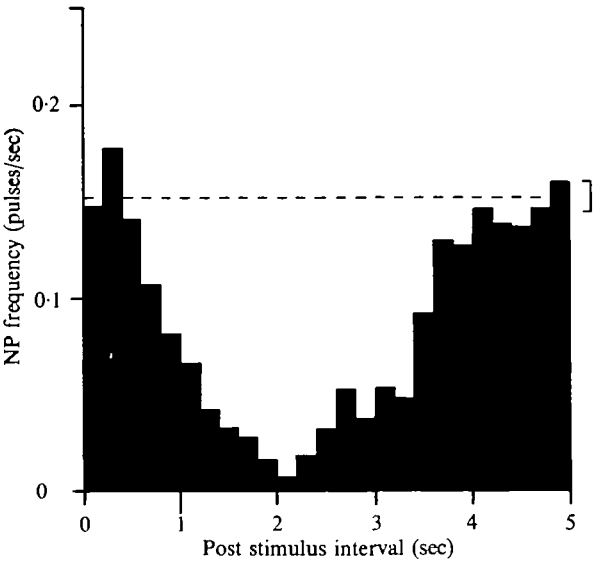

Fig. 3. The time course of NP inhibition at greater temporal resolution, based on pooled data from all stimulation periods with one stimulus every 5 sec.

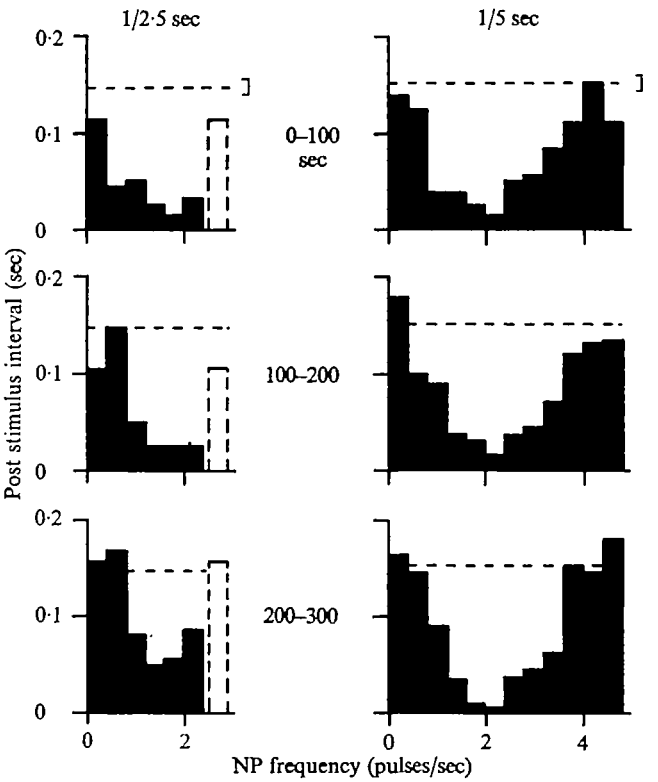

Fig. 4. Post-stimulus interval distributions for NPs during successive portions of the 5 min stimulation periods. The dotted bars in the left set of histograms are a repeat of the first bin and show the NP frequency 2·5–2·9 sec after a stimulus.

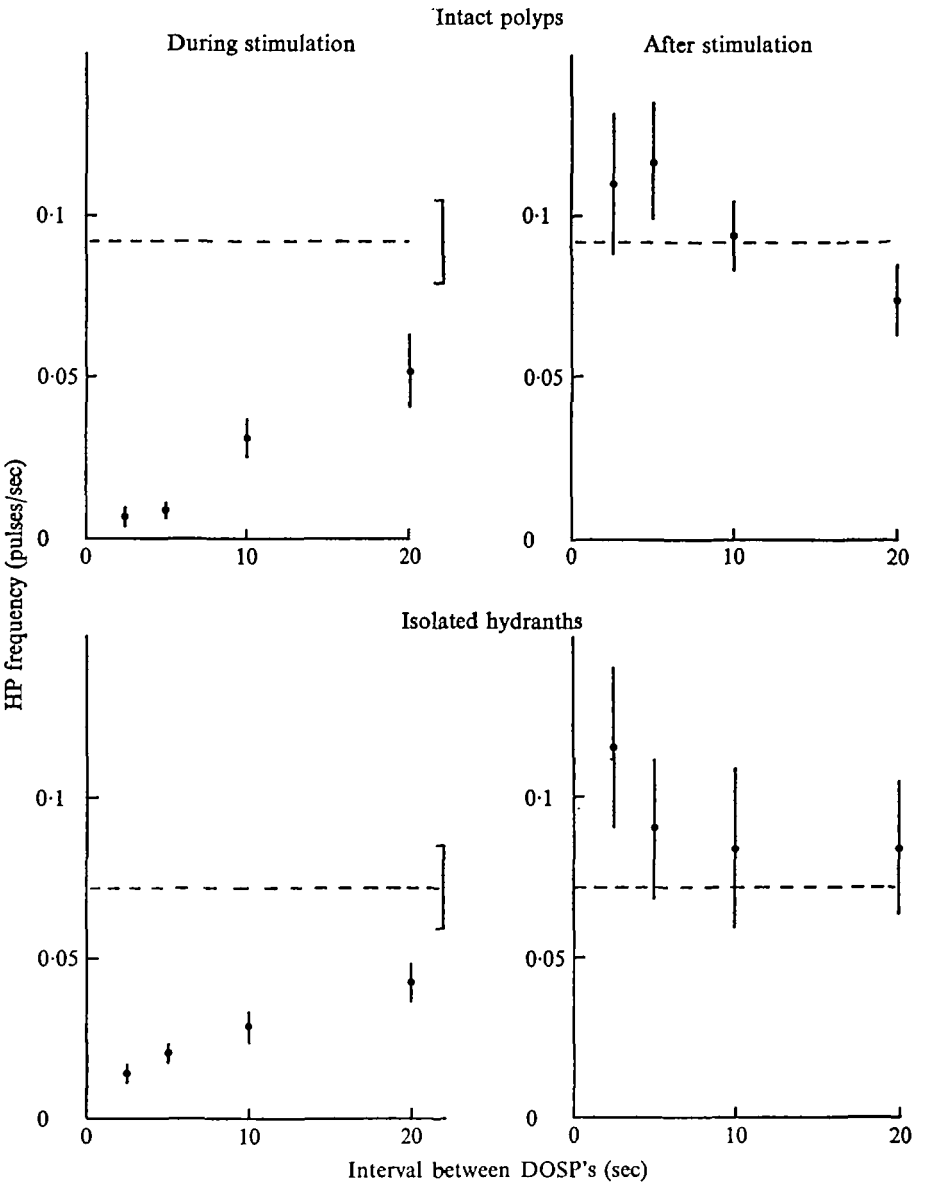

Fig. 5. HP frequency during and after DOS stimulation in intact polyps and isolated hydranths.

than in those with longer inter-stimulus intervals. At this frequency the 0 to 0·4 sec interval after a stimulus is also the 2·5 to 2·9 sec interval after the preceding stimulus. At longer inter-stimulus intervals this is still a time of pronounced NP inhibition; at 1 per 2·5 sec the NP frequency in this bin is nearly normal. The seemingly accelerated recovery from inhibition is due to diminishing effectiveness of inhibitory stimuli during the course of the stimulation period. Toward the end of the stimulation period the extent of NP inhibition after each stimulus is reduced and the recovery from inhibition is more rapid (Fig. 4). In contrast, with 1 per 5 sec the inhibition

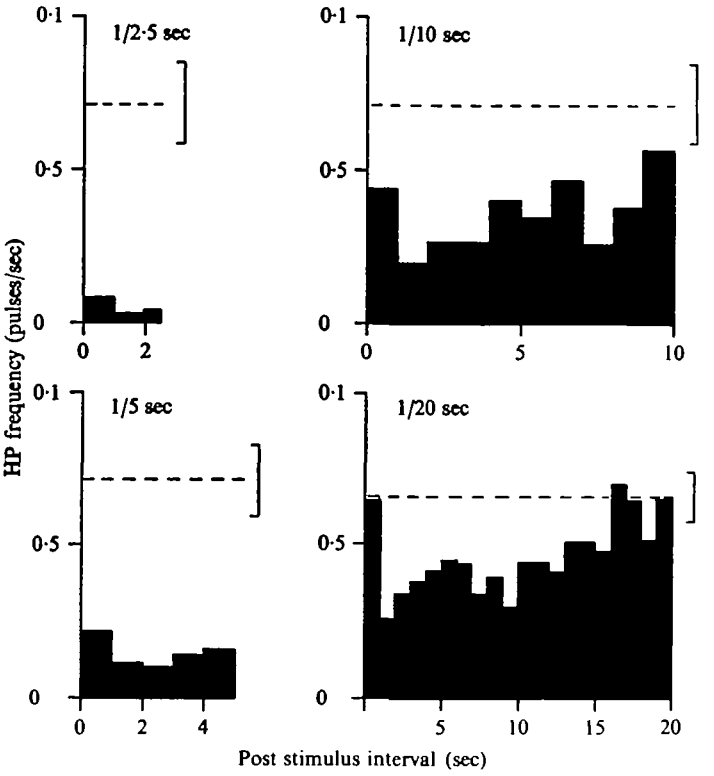

Fig. 6. The distribution of HPs during stimulation of isolated hydranths. To increase temporal resolution data from several incomplete runs has been included in the histogram with stimuli at 20 sec intervals.

following each stimulus is similar through the whole stimulation period. It appears that the declining effectiveness of inhibitory activity is due to fatigue of the inhibitory mechanism at the high stimulation frequency.

(2) *Inhibition of the HP system*

Inhibition of HP activity by the DOS is compared for isolated hydranths and intact polyps in Fig. 5. The recorded HP frequency in the pre-stimulation periods (horizontal dashed lines) is somewhat higher in intact polyps but the difference between intact polyps and isolated hydranths is not statistically significant ($P > 0{\cdot}1$). If real, the higher HP frequency in intact polyps may reflect excitatory interactions between the HP and NP systems. HP inhibition is similar in intact polyps and isolated hydranths. In both, the inhibition is greater the greater the frequency of DOS activation. The percentage inhibition of HPs is considerably greater than that of NPs, and post-inhibitory rebound following high-frequency DOS activity seems more pronounced for HPs than NPs.

Isolated hydranths were chosen for more detailed analysis of HP inhibition because with these there are not the complications arising from simultaneous NP inhibition and the effects of this on HP activity. Post-stimulus interval histograms for HPs from isolated hydranths are shown in Fig. 6. The first, second and third stimulation periods

with shocks at 1 per 5 sec gave similar histograms, indicating that there were no systematic changes in HP activity during the stimulation regime. The data from these three periods have been pooled in Fig. 6.

HP inhibition clearly last much longer than NP inhibition. Only with 20 sec intervals between stimuli did the HP frequency return to normal in the inter-stimulus interval, total recovery occurring 15–20 sec after the stimulus. The degree of inhibition in the few seconds following a stimulus is considerably greater at higher stimulus frequencies, indicating that the inhibitory effects of a stimulus can sum with those of preceding stimuli to produce more intense inhibition.

DISCUSSION

The method used to map the time course of inhibition, assembling post-stimulus interval histograms, is indirect. The events recorded, HPs and NPs, are essentially all-or-nothing pulses occurring at a low spontaneous frequency. To reliably determine the pulse frequency in a post-stimulus interval of usefully small duration requires a large sample size, acquired in these experiments by long recording sessions and by pooling results from a number of animals. The data used can be heterogeneous, both because of their origin from different animals and because of variation in individual sources. An instantaneously measurable parameter of inhibition such as membrane potential would be preferable but it is not known how this could be obtained in *Tubularia*. A curve connecting the tops of the histogram bars and inverted gives the average inhibition as a function of time; but, it should be pointed out, the shape of this curve need not correspond to the time course of inhibition in any individual case. For example, suppose that the inhibition were an all-or-nothing phenomenon, being total for some time after a stimulus and negligible at other times. If the duration of the inhibitory period were to vary, results like those of Fig. 3 could be obtained. A reduction of the pulse frequency by, say, 50% in an interval would, in this case, result from total inhibition during this period in half the trials and no inhibition at all in the other half. Although the time course of inhibition suggested by the histograms may not correspond to that following any given stimulus, it is none the less the time course of the average inhibition and thus a significant parameter.

The most striking features of the pulse inhibition are its long duration and latency. The duration of the inhibition is 4–5 sec for NPs and 15–20 sec for HPs. NP inhibition does not begin until 0·4–0·6 sec after a stimulus (Fig. 3). The latent period for HP inhibition cannot be given precisely but it appears to be even longer than for NPs (compare 20 sec inter-stimulus interval portions of Figs. 2 and 6). Part of the latency is due to conduction time from the site of stimulation to the pacemakers. The conduction velocity of the DOS in the polyp is approximately 10 cm/sec (Josephson, 1965). The locations of the pacemakers in the polyp which initiate HPs and NPs are not known, but the distance between the pacemakers and the point of stimulation cannot be more than 1 mm. Thus at most, 100 msec of the inhibitory latency can be due to conduction time in the DOS.

A number of slow inhibitory phenomena have been described in other animals, including several examples of slow, inhibitory synaptic potentials. Among the many kinds of synaptic interactions described for *Aplysia* are inhibitory synaptic potentials lasting seconds or even minutes (e.g. Tauc, 1969). Slow IPSPs with latent periods as

long as 100 msec have been recorded from frog sympathetic ganglion cells (Libet, Chichibu & Tosaka, 1968). An even closer parallel to the time course of inhibition in *Tubularia* is that seen during vagal inhibition of the frog heart. The hyperpolarizing potential following vagal stimulation occurs after a latency of 0·5 sec and is 5–7 sec in duration (del Castillo & Katz, 1955).

During long stimulation periods the overall inhibition of HPs and NPs increases with increasing stimulus frequency. The extent and time course of NP inhibition following each stimulus is independent of the stimulus frequency, except at high frequencies where there is apparent fatigue of the inhibitory mechanism. Increasing NP inhibition with increasing stimulus frequency is due simply to an increase in the proportion of the time that the NP system is fully or partially inhibited. The duration of HP inhibition following a stimulus is very much longer than that for NP inhibition, and even at relatively low stimulus frequencies the inhibition resulting from a stimulus begins before the effects of the previous stimulus have decayed. There is then summation of the inhibition, so the maximum inhibition reached after a stimulus is greater when the stimuli are close together than when they are widely spaced. Because of the long inhibitory duration and summation of the inhibition, the percentage inhibition of HPs at any frequency is greater than that for NPs. This has interesting behavioural consequences. HPs are associated with behavioural events termed 'concerts' (Josephson & Mackie, 1965). A concert involves synchronized tentacle elevation followed by a peristaltic wave beginning at the tip of the proboscis and moving basally. NPs are not directly correlated with overt behaviour; but, because of loose excitory coupling between NPs and HPs, NP firing can indirectly produce concerts by activating the HP systems. During feeding the DOS is activated and concerts are suppressed; the relation between the two events is probably causal (Rushforth, 1969). The significance of this may be that food ingestion is impeded by concerted tentacle movements or by the proboscis contraction involved in peristalsis. It thus seems important that DOS inhibition is more effective with HPs, which are directly related to concerts, than with the NPs, which are only indirectly coupled to overt behaviour.

SUMMARY

1. Stimulating the DOS, a conducting system in *Tubularia*, inhibits spontaneous firing of the principal pacemaker systems of the polyp, the NP and HP systems.

2. The overall inhibition is greater the greater the stimulus frequency and there is rebound from inhibition at the end of the stimulation period.

3. The latent period for NP inhibition is 0·4–0·6 sec and the duration of inhibition is 4–5 sec.

4. The time course and extent of NP inhibition is independent of stimulus frequency except at high frequency where there is diminishing effectiveness of inhibition with time.

5. HP inhibition has a similarly long latent period and even longer duration, lasting 15–20 sec.

6. HP inhibition from successive stimuli can sum to produce a more intense effect.

7. The behavioural significance of the inhibition seems to be to reduce spontaneous polyp movements during food capture and ingestion.

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REFERENCES

BALL, E. E. (1971). Electrical activity and behavior in the solitary hydroid, *Corymorpha palma* Torrey. Ph.D. Thesis, University of California, Santa Barbara.

DEL CASTILLO, J. & KATZ, B. (1955). Production of membrane potential changes in the frog's heart by inhibitory nerve impulses. *Nature, Lond.* **175**, 1035.

HOFMAN, F. & RUSHFORTH, N. B. (1967). Electrical activity of isolated parts of *Tubularia*. *Biol. Bull. mar. biol. Lab., Woods Hole* **133**, 469.

JOSEPHSON, R. K. (1965). Three parallel conducting systems in the stalk of a hydroid. *J. exp. Biol.* **42**, 139–52.

JOSEPHSON, R. K. (1967). Conduction and contraction in the column of hydra. *J. exp. Biol.* **47**, 179–90.

JOSEPHSON, R. K. & MACKIE, G. O. (1965). Multiple pacemakers and the behaviour of the hydroid *Tubularia*. *J. exp. Biol.* **43**, 293–332.

JOSEPHSON, R. K. & UHRICH, J. (1969). Inhibition of pacemaker systems in the hydroid *Tubularia*. *J. exp. Biol.* **50**, 1–14.

LIBET, B., CHICHIBU, S. & TOSAKA, T. (1968). Slow synaptic responses and excitability in sympathetic ganglia of the bullfrog. *J. Neurophysiol.* **31**, 383–95.

MACKIE, G. O. (1965). Conduction in the nerve-free epithelia of siphonophores. *Amer. Zool.* **5**, 439–53.

MACKIE, G. O. & PASSANO, L. M. (1968). Epithelial conduction in hydromedusae. *J. gen. Physiol.* **52**, 600–21.

RUSHFORTH, N. B. (1969). Electrophysiological correlates of feeding behavior in the hydroid *Tubularia*. *Am. Zool.* **9**, 1114.

TAUC, L. (1969). Polyphasic synaptic activity. In *Progress in Brain Research*, vol. 31 (ed. K. Akert and P. G. Waser). Amsterdam: Elsevier.