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OBSERVATIONS ON THE MYO-NEURAL PHYSIOLOGY OF A POLYCLAD FLATWORM: INHIBITORY SYSTEMS

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INTRODUCTION

In a general investigation of the myo-neural physiology of the polyclad worm, *Planocera gilchristi* Jacubowa, Gruber & Ewer (1962) reported that the response of the longitudinal parapharyngeal musculature of a decerebrate animal to direct electrical stimulation was very variable. This variability they tentatively attributed to variation in the level of excitability of their preparations. In a further examination of this problem a number of effects which appear to be inhibitory in nature have been encountered and these results partially explain the phenomena which Gruber & Ewer described. The presence of an inhibitory system in the Turbellaria, although not directly demonstrated before, has been inferred from observations of polyclad behaviour under the influence of strychnine (Moore, 1918) and from records of the behaviour of decerebrate *Planocera* (Gruber & Ewer, 1962). Inhibitory systems have also been found in coelenterates (Ewer, 1960; Horridge, 1955), ctenophores (Horridge, 1968) and indications of inhibitory control of ciliary beat in nemertines (Friedrich, 1933) are known. Thus inhibition has been demonstrated or suspected in the lowest metazoa and clear indications of the phenomenon in the Turbellaria are of no surprise.

METHODS

The methods of handling the worms and of recording and stimulating were the same as those previously described (Gruber & Ewer, 1962). Briefly, a whole animal or fragment of one was ligatured with foam plastic collars close to the posterior and anterior ends. Stimulation was by wick-electrodes attached to the collars. Stimuli were negative-going square-wave pulses. Recordings of longitudinal contractions were made with isotonic frontal levers writing on a smoked kymograph drum.

RESULTS

(i) *The effect of spontaneous activity upon the response*

It had been suggested that the variability of response to electrical stimulation could be attributed to variation in the excitability of the preparation. Preparations of the parapharyngeal muscles of both intact and decerebrate animals show spontaneous activity. On the assumption that the level of such spontaneous activity reflects the general excitability of the preparation the response of the preparation to a standard

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stimulus was studied in relation to the time since the end of a burst of spontaneous activity. In these preparations the spontaneous activity is irregular and variable in duration: thus it is difficult to decide precisely when spontaneous activity may have temporarily stopped and impossible to know when it may start again. Nevertheless, by close observation of the activity of a preparation it is possible to recognize the end, or at least the waning, of an outburst of activity. It was found that if a standard stimulus was applied to the preparation immediately after such activity was judged to have ceased, the response obtained was almost constant in size for that particular preparation. Such a response was therefore taken as a reference against which to assess others. When a standard stimulus was applied at different intervals after the cessation of activity and compared with the reference contraction it was found that the size of the

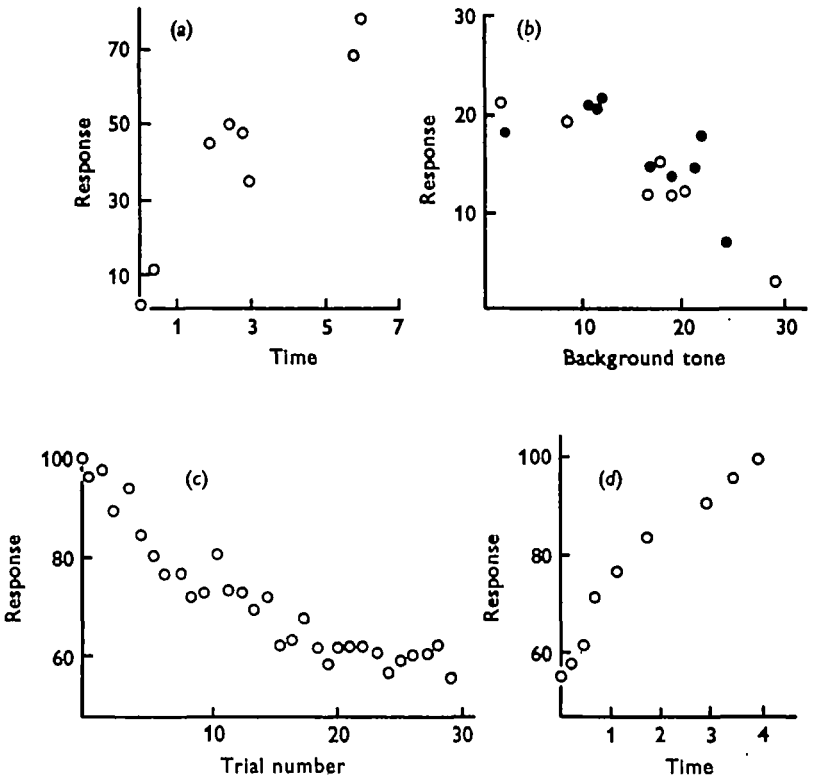

Fig. 1. (*a*) Increase in size of response with increased time since previous spontaneous contraction. Ordinate is percentage increase compared to a standard stimulus delivered directly following the spontaneous activity. Abscissa: time in minutes since spontaneous activity ended. (*b*) Changes in response with background tone of the preparation. Ordinate: response measured as mm. shortening of the preparation. Abscissa: background tone measured from an arbitrary base line and expressed as mm. between the base line and the position of the lever prior to stimulation. Data from a single preparation. Solid circles: summated response from two stimuli 1·0 sec. apart. Open circles: summated response from two stimuli 0·5 sec. apart. (*c*) Decrease in size of response with repeated bouts of stimulation. Each bout consisted of four shocks delivered at 1 sec. intervals. Interval between trials was 3 min. Ordinate: percentage change in amount of shortening compared to a standard stimulus. Abscissa: trial number. (*d*) Increasing size of response with increases in interval since stimulation. Mean data from three preparations. Ordinate: percentage increase in amount of contraction compared with standard. Abscissa: minutes since previous stimulus.

response tended to increase with time. This effect is illustrated in Fig. 1 *a*, which shows results taken from a single preparation. Despite some variability the general trend is clear. If the stimuli are applied during a period of spontaneous activity, the magnitude of the response obtained decreases with increasing tone of the preparation. This can be seen in the results presented in Fig. 1 *b*, where tone was measured from an arbitrary base line. Again, despite considerable variability the general trend is apparent.

If the experimental procedure is changed and constant stimuli are applied at regular intervals then the level of spontaneous activity of the preparation decreases (Fig. 2 *a*, *b*).

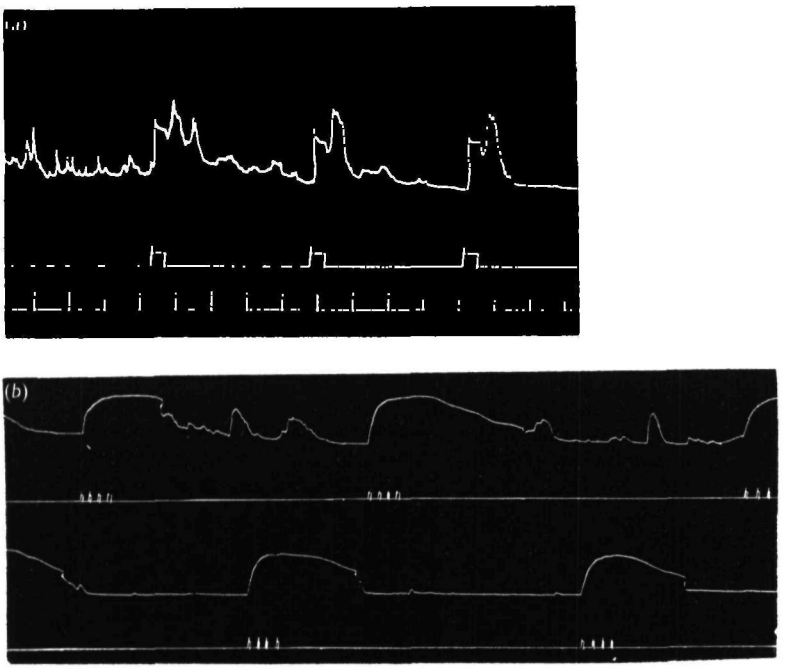

Fig. 2 (*a*) Quiescence of spontaneous activity with repeated stimulation. Stimuli were fifteen shocks at intervals of 1 sec. Upward deflexions of trace are due to contraction in this and all following traces. (*b*) Similar to the above but with a different stimulating regime. Four impulses at 1 sec. intervals. Three minutes between stimuli. The trace was speeded up during stimulation. Bottom trace is a continuation of the top trace.

Since spontaneous activity of the preparation can be almost completely suppressed by repetitive stimulation, a closer study can be made of responses to electrical stimulation. More especially, it is possible to determine the effect of one stimulation upon the response to a second. Preparations were excited with constant-size stimuli at controlled intervals of time. The results of one such experiment are shown in Fig. 1 *c*, where the effect on response size of stimuli repeated regularly at 3 min. intervals can be seen. A gradual depression of response occurs. The reverse effect is also true. If the interval between stimulations is increased, the size of the response increases (Fig. 1 *d*). If the interval between stimulations is increased beyond 5 min. no further increase in response is obtained. It is further to be noted that stimulation may evoke secondary activity from a preparation. This can be seen in Fig. 2 *a*. If the programme of repeated stimulation is maintained, this activity dies away as the magnitude of the initial response decreases.

(ii) *The effect of increased frequency of stimulation on the response*

When a preparation is stimulated at a low frequency of 1 impulse/sec. and this is abruptly increased to 5 impulses/sec. the tone of the preparation decreases. On cessation of stimulation there is commonly an increase in tone before relaxation (Fig. 3*a*). It is clear that the higher frequency of stimulation is less effective in maintaining tonus than the lower. This is true in both cerebrate and decerebrate preparations. The higher frequency is also less effective in the initial development of tone.

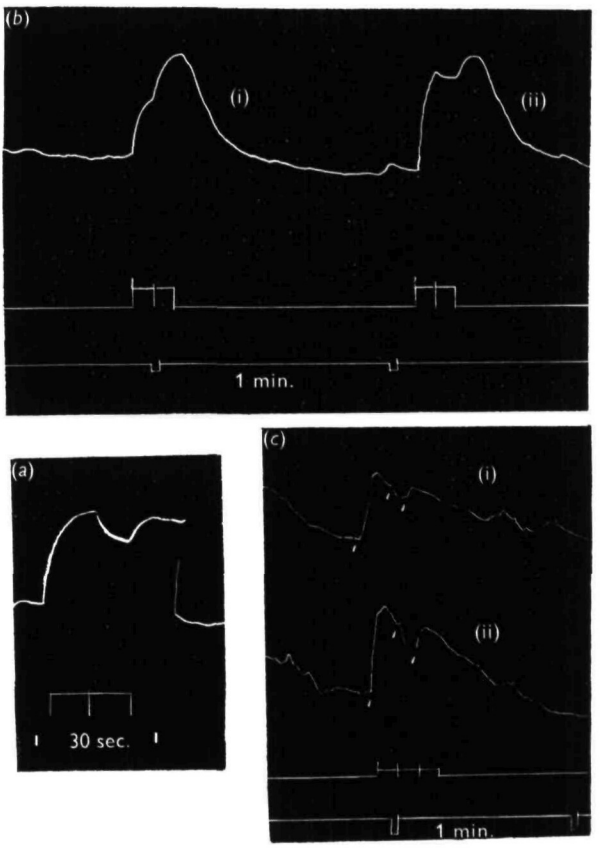

Fig. 3(*a*) Initial low-frequency stimulus 1 shock/0·6 sec.; high-frequency stimulus 1 shock/0·1 sec. Trace stopped during the rebound until the preparation had relaxed to the resting zone. Stimulated for 10 sec. at each frequency. (*b*) Initial stimulus frequency 1/0·2 sec. followed by 1/0·5 sec. (ii) stimulus frequency 1/0·5 sec. followed by 1/0·2 sec. Stimulated for 5 sec. at each frequency. (*c*) (i) Record from a witness half of a cerebrate preparation. (ii) Record from the stimulated side of the same preparation. Initial stimulation at a frequency of 1/0·7 sec for 10 sec. High-frequency 1/0·2 sec. for 5 sec. Final frequency same as initial frequency.

This can be seen in Fig. 3*b*, where the higher frequency is administered before the lower. It is clear that the higher frequency of stimulation produces a smaller initial response although the number of shocks delivered is greater.

In some preparations subjected to continuous low-frequency stimulation the contraction rises to a maximum and then starts to fall. If the frequency of stimulation is

then abruptly increased there is a strongly marked increase in the rate of relaxation (Fig. 3*c*).

Fig. 4*a*, *b* shows two records in which tone is initiated in both by the same low frequency of stimulation, but the subsequent frequency of stimulation is higher in the latter than in the former. The higher frequency of stimulation is associated with the greater loss of tone. The frequency of initial stimulation also affects the response. This may be seen in Fig. 4*c*, *d*. The higher initial frequency is followed by a greater loss in tone. A similar result is to be seen in Fig. 4*c*, where the duration of the impulses of the low-frequency stimulus is 42 msec. compared to 20 msec. in Fig. 4*f*. Relaxation only

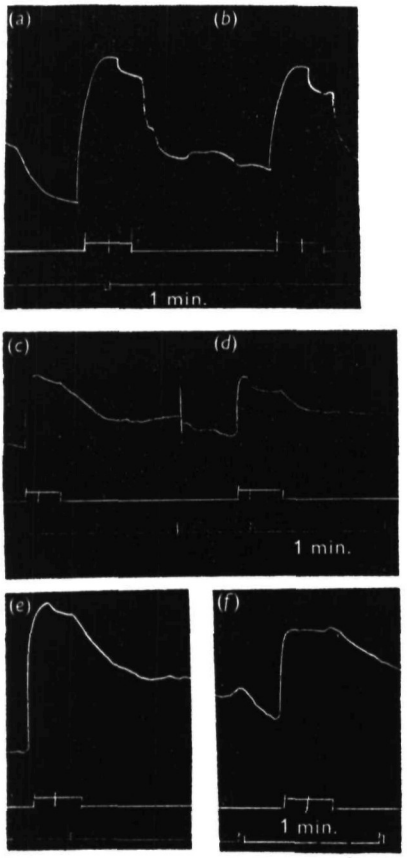

Fig. 4

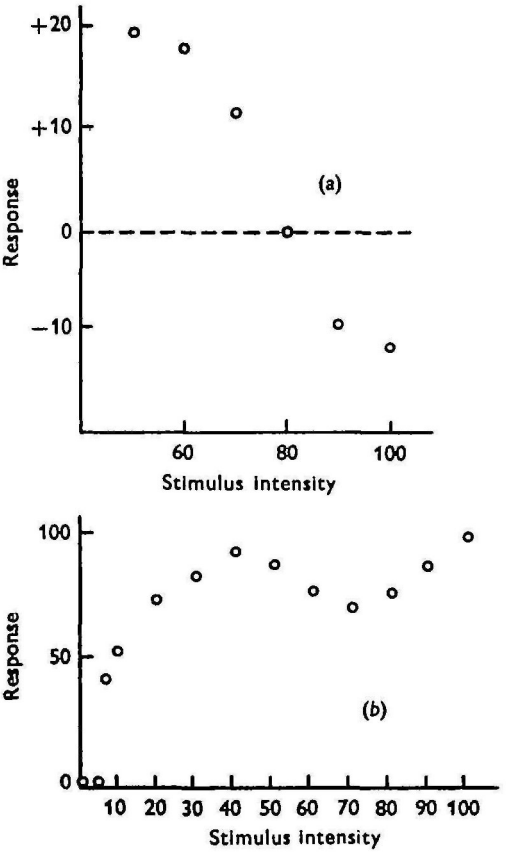

Fig. 5

Fig. 4(*a*) Initial frequency 1 shock/0·3 sec. High frequency 1 shock/0·2 sec. (*b*) Initial frequency 1 shock/0·3 sec. High frequency 1 shock/0·1 sec. (*c*) Initial frequency 1 shock/0·6 sec. High frequency 1 shock/0·1 sec. (*d*) Initial frequency 1 shock/0·2 sec. High frequency 1 shock/0·1 sec. (*e*) Initial frequency 1 shock/0·7 sec.; duration per shock 42 msec. High frequency 1 shock/0·1 sec.; duration per shock 20 msec. (*f*) Initial frequency 1 shock/0·7 sec.; duration per shock 20 msec. High frequency 1 shock/0·1 sec., duration per shock 20 msec.

Fig. 5(*a*) The response to the high-frequency stimulus at different intensities of stimulation. Ordinate: response expressed as percentage of the summation measured from the low-frequency stimulus at that particular intensity. Positive values indicate contraction and negative values indicate relaxation. Abscissa: relative stimulus intensity. (*b*) Summation of response to high-frequency stimulus only, at different intensities of stimulation. Ordinate: response expressed as percentage of the maximum contraction measured. Abscissa: relative intensities.

occurs in the former case when the high-frequency stimulus impulses are 20 msec. in both cases.

At low intensities of stimulation the effect is not shown. Fig. 5*a* shows the relative change in response at different intensities of stimulation on changing from low-frequency to high-frequency stimulation. In this particular preparation relaxation was only displayed at stimulus intensities greater than 80 V., but it is clear from the form of the curve that the effect which is ultimately expressed as a relaxation has a threshold, for this preparation, of 50 V. or less. A more direct experiment, in which the magnitude of the response at different intensities was measured, showed that above a certain value the response became less with increasing intensity of stimulation (Fig. 5*b*). A subsequent increase in the magnitude of the response at high intensities of stimulation was seen and is, perhaps, due to direct stimulation of the muscle.

It has previously been shown that excitation may pass from one side of a partially hemisected preparation to the other (Gruber & Ewer, 1962). That is, if a worm is split in the midline from its posterior margin and the cut is carried forwards as far as the anterior margin of the pharynx, stimulation of one half of the preparation is followed by a response in the other. This is only true provided that the brain has not been destroyed. In the same manner a fall in tone following high-frequency stimulation of one half of a worm is matched by a comparable relaxation of the unstimulated half (Fig. 4*c*). To test whether there is any such effect in the absence of the brain, one side of a decerebrate, hemisected preparation was stimulated at a low frequency; a high frequency stimulation was then applied to the other half of the animal. This produced no effect on the side being stimulated at the lower frequency. It would appear therefore that any pathways involved pass by way of the brain.

(iii) *Direct inhibition*

Gruber & Ewer (1962) reported that, in one preparation, single-shock stimulation resulted in an immediate loss of tone rather than in a contraction.This simple, direct inhibition has been found in three more preparations. It is now clear that the phenomenon is only displayed by preparations which have a high level of spontaneous tone, and that once this is lost stimulation will cause a contraction and not a relaxation. Despite the difficulty in evoking the event this inhibition appears to be similar to that of high-frequency inhibition. Fig. 4*f*, where the preparation does not achieve the same level of tone as in Fig. 4*e*, does not show a relaxation although the stimulus is followed by a rebound, suggesting that inhibition was evoked.

DISCUSSION

The original aim of this investigation was to determine the cause of the variability of the response obtained by Gruber & Ewer (1962), whose experiments were designed to test for neuro-muscular facilitation in *Planocera*. That the variation in the response of these preparations depends on their previous history is now clear and this is sufficient to account for the decreases in total summation reported by Gruber & Ewer, in whose experiments no attention was paid to the time interval between stimulation.

The present experiments have revealed a number of other effects which have to be considered. Characteristically, frequent stimulation leads to a fairly rapid dying away

of spontaneous activity (Fig. 2) and slower fall in the magnitude of the response to a standard stimulus (Fig. 1*c*). Such an effect could be due to fatigue, but it might also be due to some inhibitory-type phenomenon of relatively long duration. That fatigue is an unlikely explanation follows from the fact that a preparation can hold a steady tone during continuous stimulation for at least as long as $3\frac{1}{2}$ min. and only then followed by a very slow release of tone over a period of many minutes. Furthermore the fact that spontaneous activity ceases long before there is any noticeable decline in responses to electrical stimulation suggests that a simple explanation will not suffice. The effects of repeated stimulation on spontaneous activity and the possible interactions of this long-term inhibition with pacemaker centres controlling spontaneity have already been discussed elsewhere (Ewer, 1965).

The nature of the response shown to high-frequency stimulation which we have called 'short-duration inhibition' has also to be considered. Such an effect cannot be attributed to fatigue for the reasons outlined above. A second possibility is that it might be attributable to a long refractory period, so that at a high frequency of stimulation the excitable elements responded only to every other or every third shock. As a result the number of effective stimuli would be less than actually delivered. This explanation seems unlikely for, if the intensity of initial stimulation is increased, there is an increase in the extent of relaxation. Were the effect attributable to some refractory phenomenon, the opposite result would be expected. The possibility must also be considered that the phenomenon is a result of either transmitter depletion or of maintained depolarization due to liberation of excess transmitter at neuro-muscular or synaptic junctions. There are a number of reasons for excluding these interpretations. First, the effect appears to have a definite threshold lying above that of the excitatory threshold (Fig. 5). Secondly, if in experiments used to demonstrate this effect the stimulation frequency is decreased following high-frequency stimulation there is an immediate increase in tone. Lastly, the presence of a rebound after the ending of stimulation (Fig. 3*a*) is not to be expected. We therefore conclude that we are dealing with a true inhibition. This inhibitory system shows frequency-sensitive facilitation in so far as the effect is more marked at higher frequencies of stimulation (Fig. 4*a*, *b*). The initial stimulation frequency also affects the response. If the initial frequency is low then the subsequent inhibition is less than that displayed when the initial frequency is slightly greater (Fig. 4*c*, *d*). This can be attributed to the initial stimulus in the second case partly facilitating the inhibitory system. Gruber & Ewer found that 'treppe' could be recorded which showed increasing amplitudes of contraction with successive shocks, and furthermore that shortening the time interval between the shocks also led to greater total summation. They could not, however, elicit this effect reliably. We have made numerous attempts to repeat these results and have never found excitatory facilitation. The phenomenon continues to be elusive. It must be unusual that an animal should possess an inhibitory system which is facilitatory but an excitatory one which is not.

SUMMARY

1. The spontaneous activity displayed by *Planocera* preparations decreased with repetitive electrical stimulation. It was also found that the amplitude of response was related to the time since previous spontaneous activity. The response decreased as the time interval between spontaneous activity and stimulus decreased.

2. When a preparation was stimulated at a low frequency then there was an increase in the tone of the preparation. If the low-frequency stimulus was then followed by a stimulus at a slightly higher frequency then there was a drop in tone caused by a relaxation of the preparation.

3. The extent of relaxation which occurred depended on parameters or the high-frequency stimulus but could be facilitated by increasing either the frequency or the duration of the initial low-frequency stimulus.

4. Pathways involved with conduction of excitation from one side of the animal to the other pass through the brain. The brain is also required for transmission of the relaxation effect.

5. It is concluded that these relaxation and depressant effects reflect the presence of a true inhibitory system which also shows facilitation.

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