

## FACILITATION IN THE RAPID RESPONSE OF THE EARTHWORM, *LUMBRICUS TERRESTRIS* L.

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### INTRODUCTION

In previous papers (Roberts, 1962*a*, *b*) the earthworm's rapid response, mediated through the giant-fibre reflex, was described and analysed. In the course of these experiments it was noticed that in the early stages of repetitive stimulation successive responses do not remain the same size but show a marked tendency to increase in magnitude. This staircase effect is particularly pronounced in fatigued preparations in which accommodation has occurred at synapses in the giant-fibre reflex and it would appear to involve a process of facilitation in the reflex mechanism.

There are few accounts of facilitation in annelids, though there are many references to it in other groups, particularly coelenterates and Crustacea. Horridge (1959) describes spatial summation in fatigued specimens of the polychaete *Harmothoe*. Budington (1902) and Botsford (1941) both recorded staircase effects in the earthworm but neither of these workers was able to say conclusively where in the neuromuscular machinery facilitation was taking place. The purpose of this paper is to investigate the location in the rapid response reflex where a process of facilitation might occur.

### MATERIALS AND METHODS

Mature specimens of the earthworm *Lumbricus terrestris* were used in all experiments. The techniques used for stimulating and for kymograph and oscillograph recording are described in a previous paper (Roberts, 1962*a*). A number of different preparations were made and these will be described in the accounts of the individual experiments in which they were used.

### EXPERIMENTS AND RESULTS

#### *The phenomenon*

Facilitation can be demonstrated by recording rapid contractions of the posterior portion of the worm in response to peripheral stimulation at the anterior end. Stimulating electrodes, fixed to the ends of a pair of flexible copper coils so that they could move freely with the preparation, were inserted into the mid-dorsal body wall at approximately segments 7 and 12. The longitudinal contractions of 20-25 segments towards the posterior end were recorded kymographically (see Roberts, 1962*a*). The preparation was stimulated with two condenser pulses of equal intensity, both just above threshold required to produce a small rapid response.

It will be seen from Fig. 1 that the response to the second of two such shocks is

considerably greater than the first, suggesting that a facilitating effect is produced by the first response or by some component of the nervous mechanism associated with it. This facilitating process might occur at various points on the afferent or efferent side of the reflex.

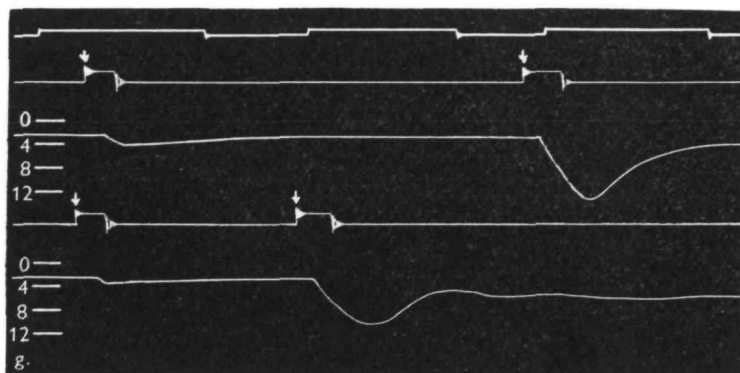


Fig. 1. *Lumbricus terrestris*. Rapid longitudinal contractions in response to stimulation of anterior mid-dorsal body wall with a pair of condenser shocks of equal intensity just above threshold for the rapid response. Two records. Stimuli arrowed. Time signal, 1 sec.

#### *Afferent side of the reflex*

Preliminary evidence that facilitation does not occur on the afferent side of the reflex was obtained by stimulating peripherally with two shocks, each delivered to a different region of the dorsal body surface at the anterior end, so that impulses set up in the median giant fibre are the result of excitation through two different sensory pathways. This eliminates the possibility that the second shock produces an enhanced response due to recruitment of additional sensory neurones or to temporal summation between sensory neurones and giant fibre.

The stimuli were supplied by a turntable stimulator (see Roberts, 1962*a*) fitted with two arms only. A simple knock-over key was used to switch the output of the stimulating circuits from one pair of electrodes to the other. The electrodes were inserted into the mid-dorsal body wall at approximately segments 6 and 30 so that they were separated by at least twenty segments. The intensity was such that a single shock, delivered through either pair of electrodes, produced a small giant-fibre response in the longitudinal muscle (Fig. 2, records *a* and *b*). The latter was recorded kymographically, a posterior region of twenty segments being connected to a light semi-isometric lever (see Roberts, 1962*a*).

The results (Fig. 2, records *c* and *d*) indicate that facilitation occurs irrespective of the point of application of the two stimuli to the body surface. The second response is enhanced whether the first shock is delivered through the anterior and the second through the posterior pair of electrodes or vice-versa.

This experiment indicates that temporal summation does not occur at junctions or endings on the afferent side of the reflex but it does not exclude the possibility that, after it has been excited, the giant fibre undergoes a temporary physiological change throughout its length, for example a lowering of the threshold, thereby enabling subsequent afferent activity to evoke in it a greater discharge of impulses than before. This

question was settled by stimulating peripherally at the anterior end and recording impulses from the nerve cord exposed a short distance behind the clitellum. The mid-dorsal body wall at the anterior end was repetitively stimulated with neon pulses at frequencies ranging from 3 to 20 per sec. In no case investigated were successive shocks found to evoke progressively larger numbers of giant-fibre impulses. Indeed the reverse was the case, the impulses declining in number and finally ceasing altogether as a result of accommodation at 'sensory-to-giant' junctions (Fig. 3). Peripheral

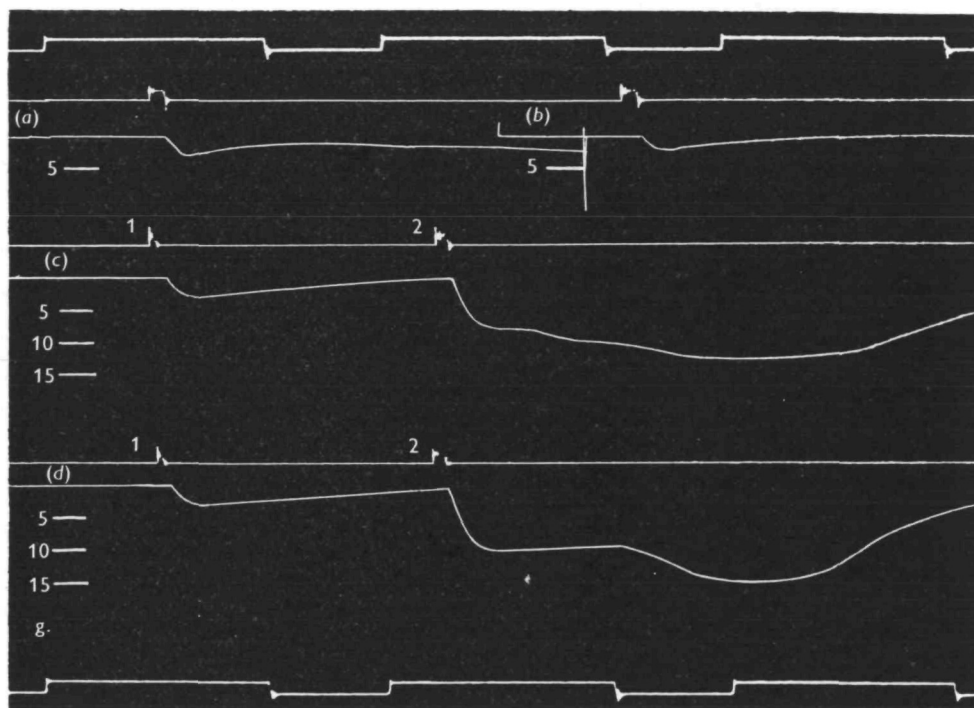


Fig. 2. *Lumbricus terrestris*. Rapid longitudinal contractions to stimulation of mid-dorsal body wall through two pairs of electrodes. *a*, Single shock through anterior electrodes; *b*, single shock through posterior electrodes; *c*, two shocks: first through posterior electrodes, second through anterior electrodes; *d*, two shocks: first through anterior electrodes, second through posterior electrodes. First and second shocks numbered in *c* and *d*. Time signal, 1 sec.

stimulation also evokes considerable activity in the slow-conducting components of the nerve cord and this, while not diminishing with repetitive stimulation, shows no tendency to increase at least in the early stages of repetitive stimulation. To find the site of facilitation it is therefore necessary to look on the efferent side of the reflex.

#### *Efferent side of the reflex*

Preliminary evidence suggests that facilitation in the rapid response is associated with the efferent side of the giant-fibre reflex. This evidence is based on the fact that a progressive increase in the magnitude of contraction still occurs if rapid contractions are recorded in response to direct stimulation of the nerve cord of a nerve-muscle preparation (for experimental set-up see Roberts, 1962*a*). The nerve cord was

stimulated at a frequency of approximately 2 shocks per sec. at an intensity which was above threshold for the small muscular response produced by an impulse in the median giant fibre but below threshold for the larger response produced by single impulses in

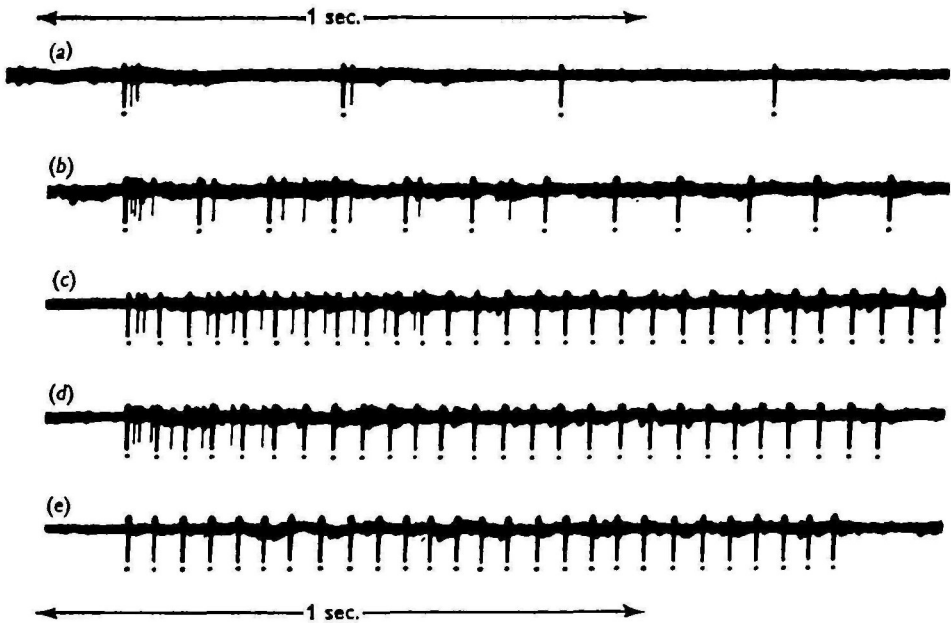


Fig. 3. *Lumbricus terrestris*. Median giant fibre action potentials in response to repetitive stimulation of peripheral nerves at four frequencies. Stimuli dotted.

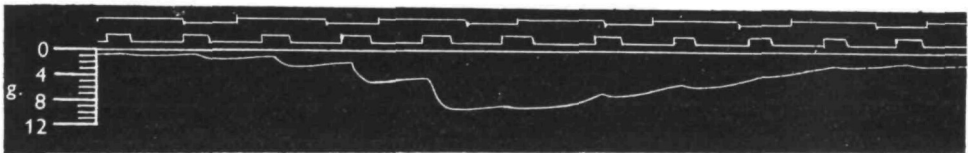


Fig. 4. *Lumbricus terrestris*. Rapid longitudinal contractions produced by repetitive stimulation of ventral nerve cord. Time signal, 1 sec.

the median and lateral giant fibres. The responses of twenty segments at the posterior end were recorded kymographically. It was found that successive shocks produced progressively larger contractions (Fig. 4). In the example shown in Fig. 4 an increase in size occurs over the first five responses, subsequent shocks producing small contractions normally associated with single impulses in the median giant fibre.

Facilitation might occur at any of the following loci on the efferent side of the reflex: (1) in the contractile machinery within the longitudinal muscle fibres; (2) at the neuromuscular junctions; (3) at junctions occurring in the course of the motor neurone tracts within the segmental nerves; (4) in the CNS (ventral nerve cord), possibly at junctions between the giant fibre and motor neurones ('giant-to-motor' junctions) or possibly at junctions within the slow system in the cord.

To ascertain the site of facilitation, experiments were carried out on the properties of these junctions and of the longitudinal muscle during the early stages of repetitive stimulation.

*Longitudinal muscle*

The longitudinal muscle fibres were investigated by recording the contractions obtained by stimulating all the muscle fibres in a short section of the body wall, twenty-five segments in length, from which the nerve cord was removed in order to eliminate effects due to giant-fibre activity. The mid-ventral longitudinal slit made for the purpose of excising the nerve cord also served to prevent effective contraction of the circular muscles. The preparations, immersed in aerated Ringer's solution and attached to a semi-isometric lever, was stimulated through a pair of Ag/AgCl plate

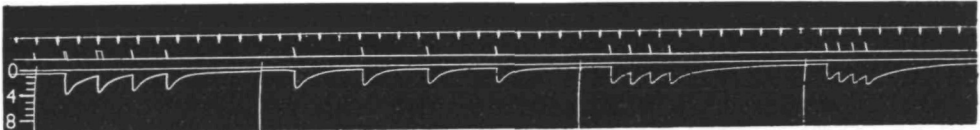


Fig. 5. *Lumbricus terrestris*. Contractions produced by direct repetitive stimulation of longitudinal muscle at low frequencies. Time signal, 5 sec.

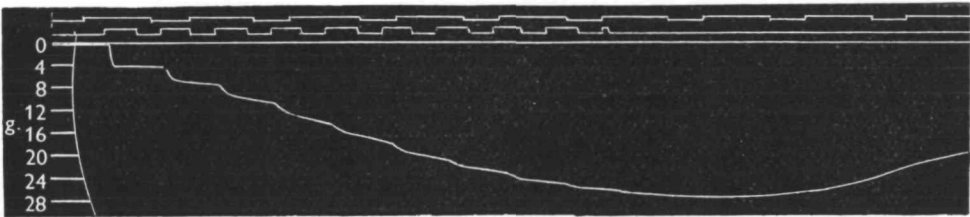


Fig. 6. *Lumbricus terrestris*. Contractions produced by direct repetitive stimulation of longitudinal muscles at 2 shocks per sec. Time signal, 1 sec.

electrodes placed on either side of it. A single shock would be assumed to excite all the muscle fibres synchronously, either directly or through their neuromuscular junctions. The preparation was stimulated with shocks at various frequencies. The contractions recorded (Figs. 5, 6) show no indication of a 'staircase', the second and subsequent responses being identical with or, at higher frequencies, smaller than the first. Clearly no facilitation process is associated with the contractile machinery of the muscle fibres.

*Neuromuscular junctions and junctions in the motor neurone pathway*

These junctions were investigated by stimulating the central ends of motor neurones in individual segmental nerves and recording action potentials in the longitudinal muscle. Details of the preparation and experimental procedure are described elsewhere (Roberts, 1962*b*). Impulses have to traverse any junctions in the course of the motor neurone pathways as well as the neuromuscular junctions before they reach the muscle. The results therefore reflect the properties of all these junctions considered collectively.

The results (Fig. 7) show that, although the first muscle potential recorded is slightly smaller than subsequent ones, an increase in the magnitude of successive responses comparable with that seen in kymograph records of successive muscular contractions (Fig. 4) does not occur. Since in this experiment the proximal ends of the

motor neurones were stimulated any junctions in the course of the motor neurone pathways, as well as the neuromuscular junctions, are eliminated as sites at which facilitation might occur. Fig. 7 shows the muscle potentials recorded in response to stimulation of segmental nerve I. Similar results were obtained with stimulation of segmental nerves II and III.

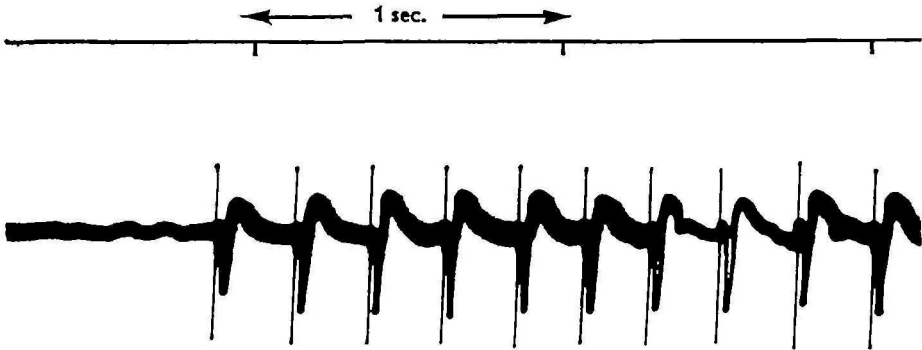


Fig. 7. *Lumbricus terrestris*. First ten longitudinal muscle potentials in response to repetitive stimulation of segmental nerve I at 4 stimuli per sec. Stimulus artifact precedes each impulse.

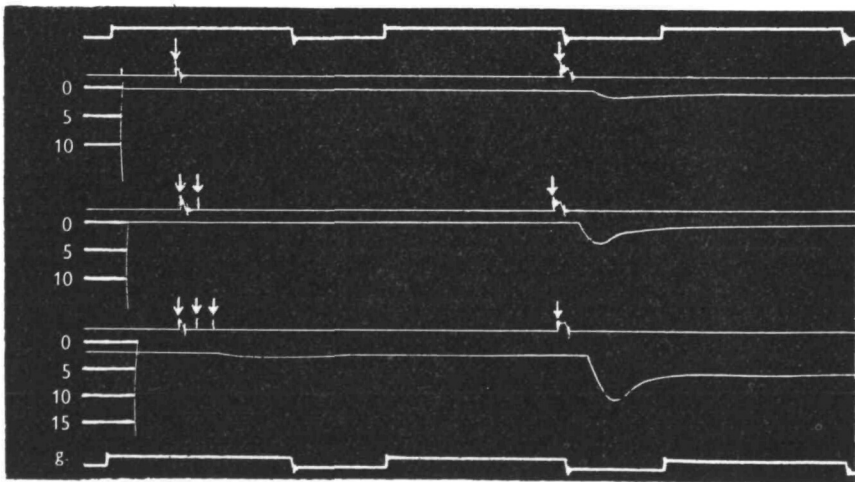


Fig. 8. *Lumbricus terrestris*. Rapid longitudinal contractions to direct stimulation of ventral nerve cord. Stimuli arrowed. Time signal, 1 sec.

### *Central nervous system*

The elimination of the longitudinal muscle, neuromuscular junctions and synapses in the motor neurone tracts leaves only the CNS as a possible site for facilitation in the rapid response. Experimental analysis of transmission phenomena in the earthworm nerve cord has not yet been undertaken, but results of experiments on the nerve-muscle preparation throw some light on events occurring in the CNS.

*Effect of varying the number of preliminary shocks*

The nerve-cord of a nerve-muscle preparation (Roberts, 1962*a*) was stimulated with condenser shocks derived from the turntable apparatus whose arms were grouped in such a way that a single shock, or a burst of two or three shocks, was followed after a known interval by a further single shock. The intensity of stimulation was such as to stimulate the median giant fibre but not the lateral giant fibres.

Fig. 8 shows the results obtained from one preparation and these are representative of many preparations studied. It will be seen that the magnitude of the facilitated response depends on the number of preliminary shocks sent into the nerve cord—that is, on the number of impulses which have previously been transmitted in the CNS.

*Size of the facilitated response and the duration of the facilitating effect*

Results of experiments on factors affecting the size and duration of the facilitating effect show considerable variation between preparations. The situation may be summarized as follows: some preparations give a greatly enhanced response to the second of two shocks (e.g. Fig. 1) whilst others produce enlarged responses only after several preliminary shocks have been delivered (e.g. Fig. 8). By stimulating with two shocks separated by varying periods it was found that a second shock can produce an enhanced contraction when it follows the first by as much as 9 sec. It therefore seems that the facilitating process decays slowly.

*The relationship between facilitation and fatigue*

A worm was anaesthetized and pinned, ventral surface downwards, to a wax block at a point just posterior to the clitellum. Fine platinum electrodes, fixed to the ends of a pair of flexible copper coils, were inserted into the mid-dorsal body wall towards the anterior end. The preparation, immersed in aerated Ringer's solution and mounted on a squared background, was allowed to recover from the anaesthetic. It was stimulated with condenser shocks derived from the turntable stimulator, at an intensity which enabled a single shock to produce a total shortening of approx 1 cm. A series of four shocks was delivered at a frequency of 1 per sec. Frequencies higher than this were not used because of complications arising from afferent fatigue (see Roberts, 1962*b*).

It was found that in a healthy and unfatigued preparation each shock in the group of four produced a rapid longitudinal contraction throughout the length of the worm. As the animal was progressively stimulated it was observed that the first of the series of four shocks evoked a response which was confined to the anterior two-thirds or so, the second shock producing a response throughout the length of the animal. With further stimulation the first shock evoked a contraction which was confined to the anterior half and later the anterior third of the body, and finally the first shock produced no response at all except for a small local contracture in the immediate vicinity of the electrodes. In all these cases the second shock produced a rapid response but, in the later stages of fatigue, this too became confined to the anterior end of the animal. In fact there was found to be a certain stage in the development of fatigue when the first shock gave no response at all, the second a rapid contraction in the

anterior end of the body, the third a contraction in the anterior and middle regions and the fourth a contraction throughout the length of the body. At the end of the experiment the nerve cord was exposed towards the posterior end of the worm and giant-fibre impulses were recorded from it in response to the same type of anterior stimulation that was used in the earlier part of the experiment.

Further experiments were designed to determine the minimum number of impulses in the median giant fibre required to evoke a rapid response. Lightly anaesthetized preparations were set up as in the previous experiment but in this case the nerve cord was exposed towards the posterior end and placed in contact with a pair of recording electrodes. The mid-dorsal body wall at the anterior end was stimulated with single shocks of progressively increasing intensity; impulses in the median giant fibre so evoked were recorded and photographed whilst the extent of the longitudinal contraction was noted. Every precaution was taken to ensure that the preparation was not in a condition of partial fatigue before the experiment. An interval of 15 min. was allowed to elapse between each stimulus and the preparation was perfused with aerated Ringer's solution throughout the experiment.

Over fifty worms were tested. In only two cases was a single impulse found to evoke a rapid contraction throughout the length of the worm. In the majority of cases a single impulse either failed to produce a response at all or, if it did so, the response was confined to the anterior end. Two impulses always evoked a rapid response but frequently this too was confined to the anterior part of the preparation. Three impulses usually produced a contraction throughout the body and four impulses never failed to do so. Larger bursts of impulses were found to produce larger muscular responses. The preparations which responded to a single impulse at the beginning of an experiment were found to require larger bursts of impulses as the experiment progressed and fatigue set in.

#### DISCUSSION

By elimination it has been shown that the mechanism causing an increase in the magnitude of successive rapid contraction resides in the CNS. The particular type of facilitation involved is therefore interneural and it is particularly marked when the animal is in a condition of partial efferent fatigue with the 'giant-to-motor' junctions accommodated.

There are few other accounts of facilitation phenomena in annelids. Budington (1902) reports on the beneficial effect of a small longitudinal contraction on the one following it, but his experimental procedure was such that it would be impossible to know through which components of the nervous system the muscle was excited. Botsford (1941), using 'whole worm' preparations and also strips of the body wall demonstrated a staircase effect when the preparation was stimulated with a series of bursts of shocks, the interval between successive bursts being varied from 7 to 27 sec. He claimed that the degree of 'augmentation' depends on the number and frequency of shocks in each burst as well as on the interval between the bursts. From his observation that 'augmentation' is enhanced by the action of physostigmine Botsford concluded that the phenomenon is the result of a persistence of acetylcholine leading to an increase in successive muscular responses. The results of my experiments, involving



stimulation with a series of single shocks rather than bursts, show little indication of neuromuscular facilitation.

In the earthworm central facilitation is most pronounced when the preparation is fatigued with 'giant-to-motor' junctions accommodated. Horridge (1959) describes spatial summation in the CNS of fatigued preparations of *Nereis* and *Harmothoë*, this being due to summation at 'sensory-to-giant' junctions which have been accommodated by previous excitation. There is considerable evidence for facilitation of previously accommodated junctions in other groups. Wiersma (1947) has described temporal facilitation at fatigued 'giant-to-motor' junctions in the crayfish, the pattern of facilitation depending on the state of the preparation. Wiersma (1949) remarks that in the crayfish different synapses are not at any one time in a similar need for summation but some will be triggered off more easily than others. Similar events are known to take place at synapses in the stellate ganglion of the squid (Bullock, 1948) and Arvanitaki (1942) has demonstrated facilitation between contiguous giant axons of the squid.

A remarkable feature of facilitation in *Lumbricus* is that the facilitating influence takes a considerable time to decay. This has not been reported on in annelids before but it is a characteristic of lower invertebrates, particularly coelenterates (Pantin, 1935; Bullock, 1943; Josephson, 1961, etc.). Josephson, investigating the transmission of impulses in the gymnoblastic hydroid *Cordylophora lacustris*, found that facilitation at interneural junctions in the nerve network can persist for as long as 10 sec. Long-term temporal summation also occurs in the luminescent response of sea pens (Pennatulacea) where apparently it is due to a combination of neuro-effector and interneural facilitation (Davenport & Nicol, 1956). Such long-lasting facilitation may be expected in slow-conducting nervous pathways but it is surprising to find it in a rapid response mechanism. However, the possibility has not yet been ruled out that the facilitatory mechanism involves the slow system in the nerve cord. The slow system requires analysis; at the moment little is known of its transmission properties and nothing is known of possible functional connexions between the slow pathways and the giant-fibre system.

Assuming that facilitation occurs at 'giant-to-motor' junctions which are in a state of accommodation it seems clear from the results of experiments on the development of facilitation during fatigue that not all the 'giant-to-motor' junctions fail to transmit simultaneously. In the case of the median giant fibre, junctions towards the posterior end accommodate and require the facilitating influence of preliminary impulses more rapidly than anteriorly situated junctions. It is logical to ask if earthworms, even in their natural habitat, are ever without some degree of efferent fatigue. It is known that a single impulse in the median giant fibre can evoke an observable rapid contraction throughout the length of the worm. However, this does not prove that every 'giant-to-motor' junction is involved in bringing about this response. Indeed recent evidence suggests that whilst some junctions transmit, others may be inactive, the degree of fatigue depending upon environmental conditions (temperature, oxygen concentration, pH and the like) as well as on the amount of activity which has previously been evoked in the giant-fibre reflex. Other cases of this kind of variability in transmission across synapses have been reported, for example in the crayfish (Wiersma, 1949) and the cockroach (Roeder, 1948). This whole question of the manner in which

environmental conditions can affect synaptic transmission requires investigation, for it is of critical importance to those who wish to study an organism's physiological characteristics as they prevail in its natural environment rather than in the artificial conditions of the laboratory.

#### SUMMARY

1. Successive rapid responses of the earthworm show a marked tendency to increase in size on repetition.

2. It is shown that this 'staircase' phenomenon is not due to peripheral facilitation either on the afferent or efferent side of the reflex, but to summation in the nerve cord and evidence is presented that it occurs at 'giant-to-motor' junctions.

3. Facilitation is most pronounced in preparations whose 'giant-to-motor' junctions are accommodated. In such cases a single impulse in the median giant fibre is ineffective, two or more being required to produce a rapid response throughout the length of the animal.

4. Fatigue and facilitation in the earthworm is discussed in relation to similar phenomena in other invertebrates.

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