Synaptic Transmission of Nervous Impulses through the last Abdominal Ganglion of the Cockroach

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[Plate 3]

In a previous communication (1936) we have described the response of the cercal nerve of the cricket when the cercus is subjected to acoustic stimuli. In the course of that work we attempted to trace the afferent fibres to their destination by recording from the ventral nerve cord at various levels anterior to the last abdominal ganglion. It was immediately apparent that, while some of the fibres from the acoustically sensitive end-organs of the cercus ran directly through the ganglion and up the cord, others terminated in the ganglion in synaptic relation with a relatively small number of fibres running forwards in the cord and yielding action potentials of considerable magnitude. Contrary to expectation we found that, subject to certain conditions noted below, the random activity in the abdominal nerve cord was never large enough to obscure the wanted signals, and it seemed to us that the preparation offered an excellent opportunity for an examination of the properties of a central nervous synapse. This paper describes the results of this examination.

The Preparation

In this work we have used the American cockroach (Periplaneta americana). As in the previous work (1936) the posterior part of the nervous system of a decapitated animal was exposed by dissection from the ventral aspect. Electrical activity in the cord which tended to mask the effects which we wished to record seemed to arise principally from two causes: (a) descending impulses originating in the thoracic ganglia and (b) injury discharges. The former could be blocked by section or ligation of the cord anterior to the first abdominal ganglion. They appeared, however, to arise

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in connexion with sensory impulses from end-organs in the legs and were generally absent when the latter were amputated close to the body. Injury discharges resulted from tension on the nervous system in dissection, from desiccation, and from extensive injury to the tracheal supply. With practice in dissection and the use of an animal which contained plenty of blood and was mounted in a moist chamber we experienced very little trouble from this cause.

Fig. 1—Sketch of posterior part of the abdominal nerve cord of cockroach (균) with the principal tracheae and the positions of stimulating and recording electrodes. The external genitalia have been removed. $R =$ recording electrodes. $S_1, S_2 =$ alternative positions of electrodes for stimulating afferent cercal nerve. $(a)$ cercal nerve (sensory); $(b)$ cercal nerve (motor); $(c)$ nerves to genitalia (cut). IV, V, VI = last three abdominal ganglia.

Fig. 1 indicates the general relations of the nervous system in the lower part of the abdomen and the positions of stimulating and recording electrodes. For the latter we have found gold wire (0.005 in. diam.) preferable to platinum as, especially after heating and quenching, it is inelastic and can be pushed and bent into any desired form.
The Apparatus

The preparation together with its recording electrode system is installed in a sound-proof, electrostatically shielded room. Stimulation, either acoustical of the anal cercus or electrical of any appropriate nerve, has been arranged respectively by means of a loud-speaker or of a separate pair of stimulating electrodes. These instruments constitute the remainder of the apparatus in the sound-proof room, all apparatus for the generation of stimuli and for the recording of responses being situated in a room entirely separate, though closely adjacent, to the latter.

The apparatus used for sound stimulation consists of a heterodyne oscillator coupled through an amplifier to the loud-speaker referred to. Attenuating networks enable control of the intensity of the stimulus over a range of 140 db. in 2 db. steps, and the oscillator is arranged to deliver approximately sinusoidal voltages at any frequency between 30 and 11,000 c./sec. A more complete description of this section of the apparatus may be found under the references given (Rawdon-Smith 1935, 1936).

For electrical stimulation we have used a double gas-discharge tube circuit, so arranged that either one or both of the tubes might be employed, in this way permitting the interpolation of rare stimuli from one tube into the regular series derived from the other. This instrument, which is conventional in circuit, is coupled by means of a special transforming device to the stimulating electrodes. Details of this will be found in an earlier paper (Pumphrey and Rawdon-Smith 1936, in Press), and it will suffice here to say that it consists of a fixed, electrostatically shielded secondary winding connected to the stimulating electrode leads, and a movable primary winding inserted in both gas-discharge tube circuits. Movement of the primary relative to the secondary permits ready control of the magnitude of the stimulus without alteration of its wave form, a prominent disadvantage of the more normal potentiometric intensity control for such a stimulating circuit. In our own case the wave form* of the stimulus, diphasic in virtue of the inductive coupling referred to, is such as to reduce to a minimum polarization effects at the stimulating surfaces. The importance of this may be noted later.

For recording purposes we have employed two identical resistance-capacity coupled triode amplifiers, of variable gain. The input circuits of these consist of the arrangement described by Matthews (1934), earth-free

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* An additional advantage of this arrangement is that the duration of the stimulus is very short (approximately 0.5 msec.). For this reason the stimulus artefact rarely obscures the response, even for very small interelectrode distances.
input leads being of great advantage when using electrical stimuli and essential when recording simultaneously from more than one point on the same animal. By the selection of suitable valves it has proved possible to abolish the resistance-capacity high-frequency oscillation suppressors there described, with some improvement of the high note response of the unit. Each input circuit and amplifier may be finally connected to a Cossor 3236 J cathode ray oscillograph, and either or both of these may be photographed on moving ciné bromide paper by means of an adapted film camera. Either or both recording systems may be used at a time, or, if necessary, one may be used for recording and the other for observation. The latter is assisted by a linear sweep device, which may be synchronized either to the stimulus or the response voltages. A description of this has been given by one of us elsewhere (Hallpike and Rawdon-Smith 1934).

RESULTS

1—Arrangement of Fibres in last Abdominal Ganglion

Before describing our experiments in detail we shall indicate the arrangement of pre- and post-ganglionic fibres, so far as we have been able to elucidate it.* The cerecal nerve proper, which may be separated from the small motor branch to the cerecal muscles accompanying it over part of its course, contains only afferent fibres. Some of these run through the abdominal ganglion into the ventral nerve cord. Stimulation, either auditory of the cercus or electrical of the cerecal nerve, elicits a response in the cord above the last ganglion, in addition to the major response discussed below, similar to that in the cerecal nerve, when due allowance is made for the greater scatter due to the increased conduction distance and the diminished amplitude due both to this factor and to the short circuiting effect of the other fibres in the cord. Our experiments indicate that most of these fibres which we shall term “through fibres” run on the same side of the cord as the cercus from which they are derived. We have not been able to trace them with certainty to higher levels of the cord.

Stimulation of the cerecal nerve of one side, however, in addition gives rise to post-ganglionic impulses of great magnitude in fibres on both sides of the cord, though there are relatively fewer of these on the contra-lateral side. These post-ganglionic fibres we shall term giant fibres from their resemblance to fibres so named in other invertebrates. By recording

* No histological examination has yet been made of the last abdominal ganglion in any Orthopteran. Our conclusions are not inconsistent with the arrangement of ganglion cells found in Aeschna by Zawarzin (1924).
R. J. Pumphrey and A. F. Rawdon-Smith

simultaneously from different points on the cord, we have shown that they run directly through the next five abdominal ganglia and impulses in most, if not certainly in all of them, can be detected in the neck just posterior to the sub-oesophageal ganglion. The arrangement which we have tentatively assumed for these fibres is indicated in the diagram (fig. 2). The through fibres may be differentiated with certainty from those synapsing in the ganglion by interchanging the stimulating and recording electrodes. A stimulus then applied to the cord will reveal, in the cercal nerve, only a minor response due to the through fibres, antidromic stimulation of the giant fibres producing no effect there.

\[\text{Fig. 2}\]

**2—Acoustic Stimulation of the Cercus**

It might be thought that, for the investigation of the response of the giant fibres to pre-ganglionic activity, acoustic stimulation of the end-organs associated with the peripheral afferent fibres would be very satisfactory, since the frequency and intensity of the stimulus could be varied with great ease and precision and, more important, one might be certain that only fibres homologous in that they are all derived from acoustically sensitive sensilla were stimulated. But synchrony of response in the cercal nerve is very imperfect at the lowest frequencies of auditory stimulation (especially in the cockroach) as some of the hair sensilla are excited more than once by each incident wave of the stimulus. For this reason we have abandoned acoustic in favour of electrical stimulation of the peripheral nerve. It is worthy of note, however, that in the cricket where synchrony of the response in the cercal nerve to auditory stimuli is more nearly perfect
we have obtained a giant fibre response synchronized with the stimulus at frequencies between 70 and 100 c./sec., and momentarily at frequencies considerably higher than this.

3—Response to a Single Electrical Stimulus of the Cercal Nerve

When the cercal nerve is stimulated by a single maximal shock and the recording electrodes are on the cord above the last ganglion, the stimulus is followed almost immediately by a response from the through fibres and, after a pause, by a closely grouped volley of impulses in the giant fibres (fig. 3, Plate 3). The pause consists in part of the conduction time and in part of the delay at the synapse. By varying the positions of the electrodes we have estimated the conduction rate in pre- and post-ganglionic fibres as approximately 1·5 and 5–6 m./sec. respectively and from this deduced a synaptic delay of about 1 msec.*

Under optimal conditions, as has been stated above, we have generally experienced little trouble from intrinsic and apparently spontaneous activity in the cord. Occasionally, however, we have observed a good deal of activity which was not associated with movements of the thorax and did not possess the very characteristic features of an injury discharge. We are inclined to regard this activity as a consequence of operative shock rather than a normal condition of the abdominal central nervous system, since it died down spontaneously if we waited. We mention it here, however, because in this hyperexcitable condition the post-ganglionic volley consequent on stimulation of the cercal nerve was very frequently followed by a pronounced after-discharge, apparently in the same fibres.

Reduction of the strength of the stimulus results in a progressive reduction of the number of giant fibres which respond and by careful adjustment it is generally possible to obtain a discharge in a single giant fibre (fig. 4, Plate 3). At intensities which are just supraliminal,† however, it is usually impossible to get a regular response to successive stimuli, owing probably to intrinsic variations in the thresholds of pre-ganglionic fibres. We have successfully reduced the number of active giant fibres to one by

* Because of the small size of the preparation, the accuracy of these determinations is probably low. By making extreme allowance for errors of observation we estimate the synaptic delay to be not less than 0·4 nor more than 2 msec. for a single pre-ganglionic stimulus.

† Terms such as this have reference to the effect of the stimulus on the post-ganglionic fibres, e.g. a just supraliminal stimulus is one which when applied to the pre-ganglionic nerve will sometimes produce a small giant fibre response. Similarly a maximal stimulus is one such that an increase in the stimulus intensity produces no increment in the giant fibre response.
dissection of the cord, but injury to the cord seems to be progressive and the life of such a single fibre preparation has so far proved too short for experiment.

4—Responses to Repetitive Stimulation

When the pre-ganglionic fibres are subjected to repeated maximal or supramaximal stimuli at regular intervals, each volley of the giant fibre response is approximately identical, provided that the stimulating frequency is low. The small variations in the grouping of the giant fibre responses we take to be an indication that the delay for each synapse is not absolutely invariant even at low frequencies of stimulation. Above a certain frequency of stimulation, there is a falling off in the total number of fibres responding to each stimulus, and, as the number of active fibres is reduced, it can be seen that the response of the remainder becomes intermittent before failing totally. At this stage it may also be seen that the interval between stimulus and response becomes somewhat longer and more variable. This increase in the interval, which may be 1–2 msec., is attributable to alteration in the synaptic delay, since the frequencies employed are not such as would cause an increase in the conduction time of either pre- or post-ganglionic fibres.

When repetitive submaximal stimuli are applied to the cerebrospinal nerve, the post-ganglionic response suffers a decline in magnitude, provided that the stimulus frequency is sufficiently high. At any given stimulus intensity, however, it is possible to find a frequency which just fails to cause such a decline. This value we have called the critical frequency, and it is clear that it is not the same for all intensities of stimulus. In our preparation, it varies from 40 to 50 per sec. for just maximal stimuli to 15 per sec. or less for submaximal. If repetitive stimuli of submaximal intensity are applied at a frequency above the critical, the giant fibre response eventually disappears almost completely. An increase in the stimulating intensity will now bring back the response, as would be expected. By recording pre-ganglionically we have satisfied ourselves that this effect is due to an increase in the number of pre-synaptic fibres excited, though there is no sign of a decrease in this number during the time when the post-synaptic response is decaying.

It is not, however, necessary to increase the intensity of the stimulus to recover the post-synaptic response, for an increase in the stimulating frequency, without modification of the intensity, is temporarily as efficacious (fig. 5, Plate 3). Investigation of the pre-ganglionic response under these conditions reveals that there is no change whatever in the amplitude or form of the action potential here, and that the only modification of this
Synaptic Transmission of Nervous Impulses

response is an increase in frequency. It appears justifiable, therefore, to regard this augmenting effect of increased stimulus frequency as having its locus in the synaptic connexions between the cerical fibres and the giant fibres of the ventral cord.

It would be deduced from the foregoing data that, during repeated stimulation, when the post-synaptic response has become almost negligible, the interpolation of an extra stimulus into those of the regular series should cause a momentary return of the response. This expectation has been found to be justified and, in fig. 6, Plate 3, it may be seen that such an additional stimulus, even though its magnitude is considerably smaller than those of the series proper, is efficacious in causing a single response to the stimulus of the regular series immediately following it. On occasions, the response secured in this way may occur to the interpolated stimulus itself (fig. 7, Plate 3), especially if this should occur immediately after one from the series. The relative sizes of the two stimuli may be noted from fig. 8, Plate 3.

In fig. 7, Plate 3, it is clearly seen that the response of the through fibres, which do not synapse in the last abdominal ganglion, is maintained throughout the experiment. In this figure the through fibre response is, relative to that from the giant fibres, somewhat greater than is customary, and it may be worthy of note that the relative sizes of the two appear to be to some extent dependent on the age of the preparation.

In all these cases it is often possible to identify, in the oscillograph figure, the responses of single fibres which, owing perhaps to some special relation to the recording electrodes, display an action potential wave of characteristic form. It is thus possible to be certain that a response which has disappeared, after repeated stimulation, reappears in the same giant fibre on raising either the frequency or the intensity of the stimulus. It follows, therefore, that some at least of the giant fibres in this insect are synaptically connected with two or more pre-ganglionic fibres.

It might also be expected that a preparation which behaved in this way would display the phenomena of temporal summation in the classical sense. This expectation is not fulfilled. It is impossible to find a frequency and intensity of stimulus such that the initial stimuli of a series are ineffective in producing a post-ganglionic response while later stimuli are successful. Indeed, it may be shown that, when a series of liminal stimuli is applied to the preparation, the response is erratic in that by no means every presynaptic stimulus yields a post-synaptic response. The first stimulus of the series, however, is as likely to yield a response as any other. This appears to be true irrespective of the stimulation frequency. Moreover, we have
not been able to demonstrate a shortening of the synaptic delay for a stimulus following rapidly on another, such as has been described for the mammalian flexor reflex (Eccles and Sherrington 1931).

Both these considerations argue against a slow building up of the excitatory state and the existence of a “subliminal fringe”. It is clear that current theories of central excitation are not directly applicable to our preparation.

**DISCUSSION**

Repeated maximal stimulation of the cercal nerve at a frequency above the critical frequency results in the giant fibres no longer being excitable through their synapses, though both pre- and post-ganglionic fibres may still be excited by direct electrical stimulation. This condition of the synapses we have designated fatigue, since it appears to be in every way comparable with the fatigue of the neural end-plates in vertebrate striated muscle.

It is of interest to contrast these observations with those of Bronk and Pumphrey (1935) on the synapses of the inferior cervical ganglion of the cat. They found that stimulation of the upper thoracic sympathetic roots at a low frequency produces a synchronized discharge in the post-ganglionic fibres of the inferior cardiac nerve. At higher stimulation frequencies, (say 80/sec.) the synchronous volleys disappear after the first few stimuli, and thereafter (with monophasic leads and a direct-coupled amplifier) the post-ganglionic response is seen to consist of a smooth negative deflexion of the base line co-durational with the period of stimulation. It was shown that pre-ganglionic stimulation was effective in maintaining the acceleration of the heart for long periods at stimulus frequencies from 60 to 120 per sec., and that, within this range, the degree of acceleration was independent of the frequency. It was concluded that at the higher frequencies the post-ganglionic fibres entered into a self-regulatory cycle of excited and refractory phases, independently of each other and of the periodicity of the incoming pre-ganglionic impulses, producing a random discharge in the post-ganglionic trunk of which the negative deflexion of the base-line represented the statistical sum. This preparation, therefore, did not show fatigue of the type which we find in the cockroach.

With repetitive submaximal stimulation the situation is more complex, and our results invite comparison on the one hand with those obtained from the study of mammalian reflexes and on the other with observations of the physiology of the elementary nerve-net of the Actinozoa.
Synaptic Transmission of Nervous Impulses

Repetitive submaximal stimulation of the cercal nerve gives rise to a condition of the synapses which we have designated “adaptation”. It has been noted that there is a fall in magnitude of the response with time during a period of repetitive stimulation at a constant intensity, but this does not prevent the preparation from responding to a change in the magnitude of the stimulus. We believe the analogy with the adaptation of sensory end-organs to be very close (cf. for example Bronk and Stella 1935).

In the adapted condition the preparation responds by an augmentation of the post-ganglionic response, not only to an increase in the intensity of the stimulus, but to an increase in frequency. The latter type of augmentation is clearly allied to the phenomenon of summation which is a widely distributed property of synapses (Sherrington 1906; Pantin 1935). It must be emphasized, however, that in our preparation summation occurs only in the adapted state. In the unadapted synapse it is quite impossible to obtain a response from a series of stimuli of such a magnitude that each alone is subliminal. As has been pointed out, the response is irregular at stimulus intensities just on the threshold but, whatever the frequency, the response, if it occurs at all, is as likely to occur to the first stimulus of a series as to any other. There is here no parallel with the phenomena of summation described by Sherrington (1906).

We believe that the explanation of these facts is as follows: the arrival at the unadapted synapse of an impulse along a single pre-ganglionic fibre is adequate to initiate an impulse in the corresponding post-ganglionic fibre. In support of this view we find firstly that on gradually reducing the intensity of a very slow series of stimuli the pre- and post-ganglionic responses disappear completely at the same intensity; and secondly that stimulation of a single pre-ganglionic fibre by agitation of the hair sensillum from which it is derived is often adequate to produce a post-ganglionic response.

It seems probable, therefore, that adaptation of the synapse consists in a prolongation of the relatively refractory state of the synaptic terminations of pre-ganglionic fibres. It may be supposed that the arrival of an impulse at a synapse from a single pre-ganglionic fibre raises what we may call the excitatory potential whether by causing the secretion of a neuro-humor or otherwise. In the unadapted synapse the potential reaches the threshold of the post-ganglionic fibre and initiates an impulse in the latter. In the adapted synapse the threshold is raised so that an impulse in a single afferent fibre fails to excite; the excitatory potential can be raised to the higher threshold by exciting more pre-ganglionic fibres or by decreasing sufficiently the interval between successive stimuli. It must therefore be
assumed that the complete decay of the excitatory potential occupies a time longer than the interval between stimuli at the frequencies at which summation occurs in the adapted preparation (i.e. at least 20 msec.). On the other hand we have reason to believe that it reaches its maximum relatively rapidly, since the synaptic delay is never longer than 3–4 msec., even in the fatigued or adapted preparation.

It must also be assumed that if stimulation of one pre-ganglionic fibre raises the excitatory potential at the synapse to the threshold, the simultaneous stimulation of several will raise it much higher. There is therefore a possibility of re-excitation of the post-ganglionic fibres when they have passed through their refractory phase. This we believe to be the most probable explanation of the after-discharge sometimes observed (p. 111).

The fact that summation is demonstrable in the adapted synapse also renders it unlikely that the initiation of an impulse in a post-ganglionic fibre “uses up” an appreciable amount of the excitatory potential.

In our discussion we have purposely avoided extending the term c.e.s. to cover our observations. This expression has already become somewhat ambiguous through its application to the phenomena observable in peripheral ganglia, and it seemed advisable to describe our results so far as possible in terms, such as fatigue and adaptation, which are already, in spite of difficulties in definition, well recognized phenomena in the physiology of the periphery. We have used the expression “excitatory potential” because, while its meaning is self-evident, it has no historic associations and implications. As has been shown it has some attributes in common with c.e.s. and others which are not.

The facilitatory effect of interpolating a single extra stimulus into those of a regular series may be compared with certain phenomena to be noted in human subjects.* If a series of regular, short-duration acoustic stimuli is applied to such a subject at an intensity sufficient to elicit the “blink reflex” response, it will be found that, under certain conditions of duration, intensity and spacing of the stimuli the response gradually dies away. It is then said to be inhibited. If, now, a stimulus of similar type is interpolated in the series a response will be obtained, occasionally to the interpolated (disinhibitory) stimulus itself, though more often to that of the regular series immediately following it. Precisely similar phenomena, as has been noted, are seen in our own case.

Attention may also be drawn to the somewhat less obvious, though none the less clear similarity between these results and certain other effects

* We are greatly indebted to Mr R. C. Oldfield for this information, and for his permission to use it here.
obtained in human subjects (Rawdon-Smith 1936). It was here shown that the application of a sustained monaural pure tone stimulus of considerable intensity led to a loss of auditory sensitivity both ipse- and contra-laterally. The ensuing application of an unexpected stimulus in the same or another sensory mode led to a recovery of contra-lateral sensitivity for a period of several seconds.

**Summary**

A method is described whereby the response in the central nerve cord to either electrical stimulation of the cercal nerve or acoustic of the cercus itself may be investigated in the cockroach.

Investigation reveals that the response in the cord anterior to the last ganglion is twofold; first, a small response due, it is thought, to the presence of a small number of fibres which pass through the ganglion without synapsing, and which have been called, therefore, “through fibres”; and secondly a response of great magnitude due to what have been called “giant fibres”. The latter, it is contended, are few in number, and there is evidence that each such fibre is in synaptic connexion with a number of fibres from the cercal nerve.

The post-ganglionic response to repetitive submaximal electrical stimuli applied to the cercal nerve exhibits a number of interesting features. At low stimulus frequencies (around 25/sec.) the response exhibits a decline in amplitude, until the majority of the stimuli fail to excite the post-ganglionic fibres, though it is shown that the pre-ganglionic response is in every way identical throughout the stimulating period. The post-ganglionic response may be brought back in one of three ways: by increasing the number of peripheral fibres stimulated, i.e. raising the stimulus intensity; by increasing the stimulus frequency, without modification of its intensity; or by the interpolation of an extra stimulus into the series. Such a stimulus yields only a momentary effect, but nevertheless produces unmistakable results even if it is of much smaller intensity than the stimuli of the series in which it is inserted.

A tentative theory is put forward to account for these phenomena.

**References**


R. J. Pumphrey and A. F. Rawdon-Smith

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DESCRIPTION OF PLATE

All records read from left to right. Time in all cases = 20 msec.

**Fig. 3**—Post-ganglionic response to single maximal pre-ganglionic stimulus. Note, from left to right, (a) stimulus artefact; (b) response of through fibres; (c) large response of giant fibres.

**Fig. 4**—Post-ganglionic response reduced to single giant fibre; note persistence of through fibres.

**Fig. 5**—Post-ganglionic response to repetitive pre-ganglionic stimulation. Note that the slow rate of stimulation, approx. 28/sec., fails to produce a post-ganglionic response, but that when the stimulating rate is raised to approx. 105/sec. a response is readily secured, though the stimulating intensity is identical in both cases.

**Fig. 6**—Post-ganglionic response to repetitive pre-ganglionic stimulation. Stimulation at 27/sec. fails to produce a response until the interpolated stimulus is applied. A large response is then secured to the next stimulus of the series.

**Fig. 7**—Similar to fig. 6, though another preparation. On this occasion the interpolated stimulus, coming shortly after one from the regular series, itself yields a response. Note the persistence of the through fibres, which respond to each stimulus.

**Fig. 8**—The relative sizes of the two stimuli used in figs. 6 and 7. The smaller of the two is the interpolated stimulus.