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EXCITATION AND HABITUATION OF THE CRAYFISH ESCAPE REFLEX: THE DEPOLARIZING RESPONSE IN LATERAL GIANT FIBRES OF THE ISOLATED ABDOMEN

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Crayfish commonly evade capture by darting backwards when one tries to pick them up or otherwise disturbs them. They do this by sharply flexing the abdomen, the tail fan acting as a paddle; at the same time they streamline themselves by thrusting their appendages forward.

Like many other escape and startle reflexes throughout the animal kingdom, this one is exceedingly labile. A crayfish held by its carapace will usually perform an escape response the first time its tail is briefly squeezed in a mock attempt at capture. But it is uncommon to find an individual which will respond to more than the first few such stimuli when these are presented at 5 min. intervals, and recovery from such waning requires a number of hours of rest (Krasne & Woodsmall, in preparation). It is the purpose of the work whose beginnings are described here to analyse the physiological mechanisms responsible for this phenomenon in the hope that an understanding of this relatively simple sort of behavioural plasticity will ultimately contribute in some measure to our understanding of how nervous systems allow for learning and memory.

The present behaviour pattern was chosen for analysis because it is commonly (Wiersma, 1947), though apparently not necessarily (D. Kennedy, personal communication), mediated by giant fibres whose position as interneurones in the reflex arc provide a convenient vantage point for looking for sites of lability. Direct stimulation of either the medial or lateral giant fibres of the nerve cord can call out the escape manœuvre reliably, though not necessarily with full vigour (Kennedy & Takeda, 1965), at least as often as once every 10 sec. for many trials (F. B. Krasne, unpublished observations). Therefore, it is not possible that the total failure of reflex responses described above can be due to changes occurring efferent to giant fibres. Consequently, it seemed appropriate to start analysis of escape reflex lability by examining reflex transmission between afferent nerves and giant axons. Wiersma (1947) had shown that it is the lateral giant fibres which can be fired in response to abdominal stimulation, and Kao & Grundfest (1956) and Kao (1960) had demonstrated that subthreshold depolarizing responses could be recorded from these fibres in their course through the abdomen. These observations were the point of departure for the work reported here.

METHODS

Procambarus clarkii about 2·5 in. long from rostrum to tip of tail were obtained from Brecia's Frog Farm (P.O. Box 3025, Compton, Calif. 90204) and maintained in groups of less than a dozen in 10 gal. aquaria filled with de-chlorinated water which was kept filtered and heavily aerated at about 21° C. Experiments were run all year round.

Prior to starting dissection of an animal it was cooled gradually to about 5° C. The abdomen was then separated from the thorax, the exoskeleton was cut through at the line of articulation of terga and pleura, and the abdomen was pinned dorsal side up on a bed of plasticine in a 100 c.c. Petri dish filled with van Harreveld's solution. The dish rested on ice which kept the physiological saline at about 8° C., and a thin slat of Perspex in the plasticine allowed for transillumination of the cord through the ventral integument. The terga, the underlying extensor musculature, and the gut were removed, and the flexor musculature was separated in the mid line to expose the nerve cord below. Care was taken to cut as little muscle as possible, and the preparation was frequently washed with jets of saline to flush away waste and aerate the bath. All the third motor roots were cut. Finally the muscle masses of the two sides were spread and held apart with a pair of pins in each segment. Once the dissection, which took about 25 min., was complete, a stream of cold, well-aerated van Harreveld's solution from a large reservoir was allowed to flow over the preparation at a rate of about 35 c.c./min. for the duration of the experiment; this kept the preparation at 12–14° C.

A pair of stimulating electrodes was placed dorsally at one end of the cord and a recording electrode at the other to monitor giant-fibre activity extracellularly. Roots which were to be stimulated were usually lifted on to pairs of closely spaced platinum hook electrodes insulated along their shanks; occasionally, stimulation was through a silver wire, tapered to 10–50 μ and insulated to very near its tip with Insl-X.

A micropipette filled with 2·5 M-KCl (or occasionally with 1 M potassium acetate), having 10 MΩ resistance and a tip which tapered rapidly to under 0·5 μ was then placed in a lateral giant fibre without de-sheathing, just rostral to the septal junction of a third or fourth abdominal ganglion. The intent was to get the electrode tip as close as possible to the large posteriorly directed process which dips ventromedially into the neuropile, sends a commissural process to the mid line, and then sends branches presumably dendritic laterally (Fig. 1). As an aid to penetration the giant fibres were stimulated directly every 2 sec.; this does not affect subsequent reflex responsiveness. The microelectrode was generally in place 1–2 hr. after the start of the dissection, and data were obtained during the next several hours. Preparations often deteriorate rapidly when kept longer.

Stimuli were 0·1 msec. pulses from a Tektronix pulse generator which were isolated from earth by an isolation transformer. For some experiments an Electronics for Life Sciences constant-current stimulator was used.

Microelectrode output was fed into a Medistor Negative Capacitance Electrometer Amplifier and gross recording electrode output into one side of a Tektronix 122 preamplifier. A coil of chlorided silver wire which was connected to earth through

the Medistor's electrode compensation voltage was placed in the bath as an indifferent electrode. In some cases high-frequency noise in the microelectrode channel was suppressed by placing a capacitor across the Medistor output; spike form was not thereby noticeably altered.

Signals were displayed on a Tektronix 502 A oscilloscope and photographed in conventional fashion. In preparing figures for publication spikes were sometimes retouched.

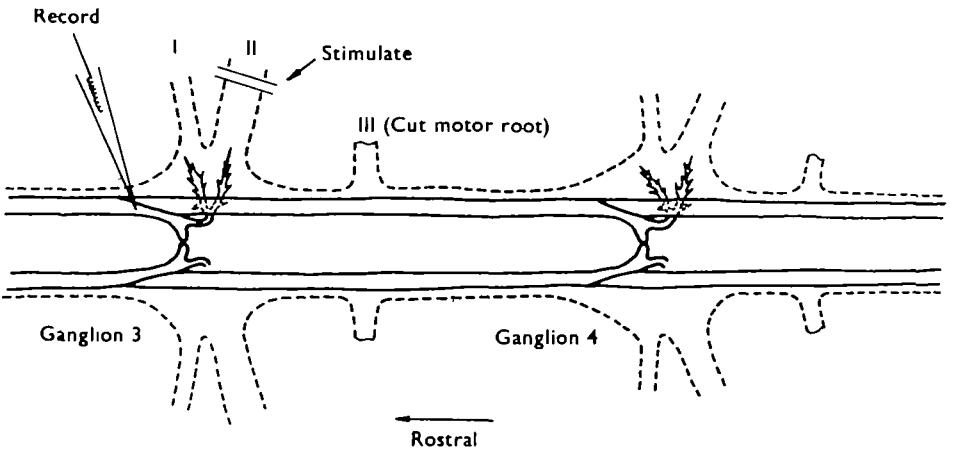

Fig. 1. Dorsal schematic view of part of the abdominal nerve cord (- - -) and its lateral giant fibres (—) with typical positions of stimulating and recording electrodes shown. (Based on Johnson, 1924.)

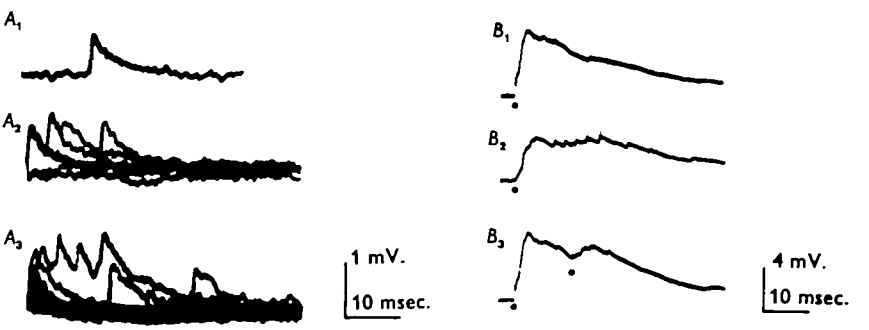

Fig. 2. Responses evoked by natural and electrical stimuli. A_1, A depolarization occurring without intentional stimulation. A_2 and A_3, Potentials evoked in the same preparation by gently tapping the table (sweeps triggered by vertical amplifier). B, Recording pipette in the fourth abdominal ganglion. B_1, The ipsilateral second root of the fourth ganglion was stimulated. B_2, The homologous root of the third ganglion was stimulated. B_3, The above inputs were stimulated in order, 12 msec. apart.

RESULTS

I. *Survey of input to the system*

A microelectrode placed in a lateral giant fibre just rostral to the septum at the level of a third or fourth abdominal ganglion in an undisturbed preparation usually records a steady potential of 85–90 mV. punctuated every several seconds by small,

brief depolarizing responses like that in Fig. 2, A_1. These potentials rise rapidly to a maximum which is variable but generally under a millivolt, and decay relatively slowly and usually monotonically. They occur in flurries if the table on which the preparation rests is lightly tapped, if one blows gently on the surface of the bath, or if one lightly strokes the exoskeleton at almost any segment of the abdomen (Fig. 2, A_2, A_3).

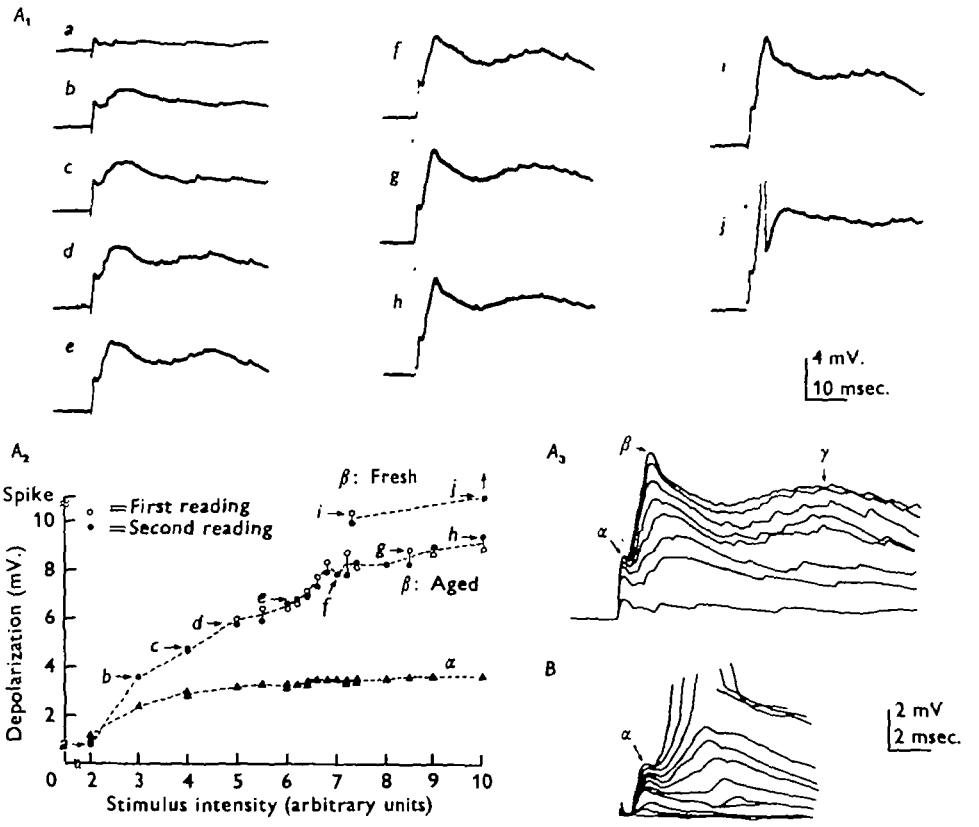

Fig. 3. The lateral giant-fibre response as a function of stimulus intensity. The stimuli were in all cases shocks applied proximally to the second root nearest the recording electrode. Preparation *A*: stimuli were given at 3 min. intervals. Traces *a–h* of A_1 are responses to a series of shocks of increasing size. Traces *i* and *j* are responses to shocks about the same size as those evoking *f* and *h* respectively, but they were obtained early in the experiment while the preparation was relatively fresh; note that an action potential occurred in *j*. Tracings of responses *a–h* have been superimposed in A_3; the three components which are evident have been labelled alpha, beta, and gamma. In A_2 the size of the alpha and beta components are graphed as functions of stimulus strength. Two consecutive measurements were made at each setting of stimulus strength. The points which correspond to traces shown in A_1 are identified. Preparation *B*; the experiment was analogous to *A* except that stimuli were applied at 30 sec. intervals. Notice that the alpha component can be graded in at least eight steps by varying stimulus intensity. (Traced from photographs.)

Large, long, and rather complex depolarizing responses can be obtained by direct electrical stimulation of segmental roots of both the impaled and other ganglia (e.g. Figs. 2B and 3). Specifically the first and second roots of the impaled ganglion ipsilateral to the recording electrode, the contralateral second root, and the ipsilateral

second roots of adjacent ganglia in both the rostral and caudal directions have been tested. All of these have yielded depolarizing responses.

Whenever the interaction of several effective afferent pathways has been tested a great amount of occlusion has been found (Fig. 2, B_3). There is nevertheless some summation, and on one occasion it was sufficient to produce a spike in a preparation which could not otherwise be made to give spikes.

II. *The response to second root stimulation*

We have analysed most intensively the responses evoked by single-shock stimulation of the ipsilateral second root of the impaled ganglion; the remainder of this account is concerned primarily with their characteristic features.

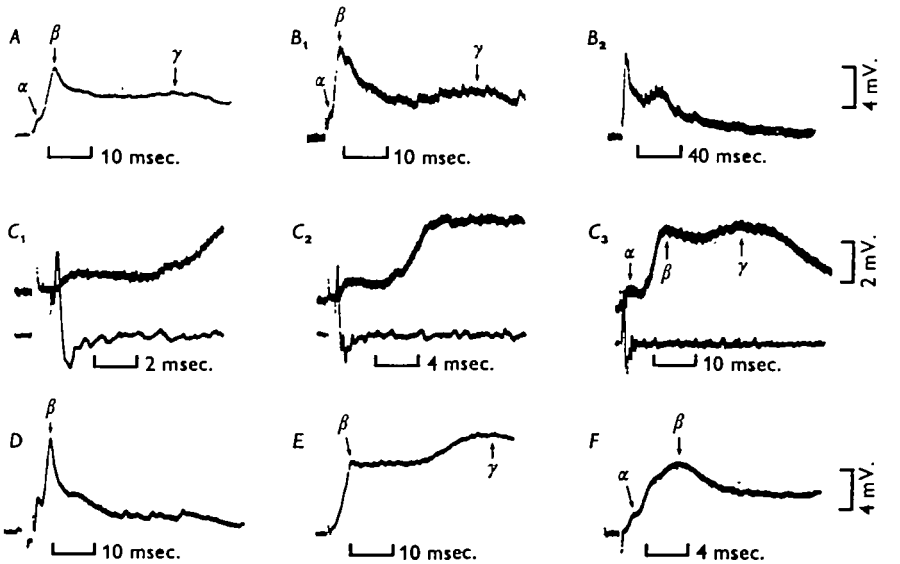

Fig. 4. The characteristic response and some special cases. Different letters denote different preparations. In each case stimulation is in the segment containing the recording electrode. *A*, Normal response to ipsilateral second root shock close to the ganglion. B_1, As above. B_2, Same as B_1, but at a very slow sweep speed to show the total course of the characteristic response. C_1–C_3, second root stimulating electrodes were placed as far distally as possible and a recording electrode whose output is shown on the lower trace was placed at the entrance of the second root into the ganglion. Notice the clear separation of the depolarizing response from the stimulus artifact and its relation to the second root volley. Notice also that while there are three peaks, they are spread out and of long latency due to the distal placement of stimulating electrodes. *D* and *E*, Atypical responses to second root stimulation. *F*, Response to ipsilateral first root stimulation.

Figure 3, A_1, illustrates the typical responses of the system to a series of well-spaced second root shocks gradually increasing in intensity. Figure 3, A_1, *i*, and *j*, are from a series early in the experiment when action potentials were easily evoked, while Fig. 3, A_1, *a* to *h* inclusive, are from one later in the experiment when they were not. We shall discuss the form of subthreshold responses first and then turn to the subject of spike initiation.

The subthreshold response to fairly large shocks is clearly tripartite in form

(Fig. 3, A_3, Fig. 4) so long as the stimulating electrodes are close enough to the ganglion so that the dispersion of impulse arrival times there is relatively slight. Since the behaviour of each of the three portions differs in its response to stimulus intensity and repetition-rate variations, we shall discuss them as discrete entities, the alpha, beta, and gamma components.

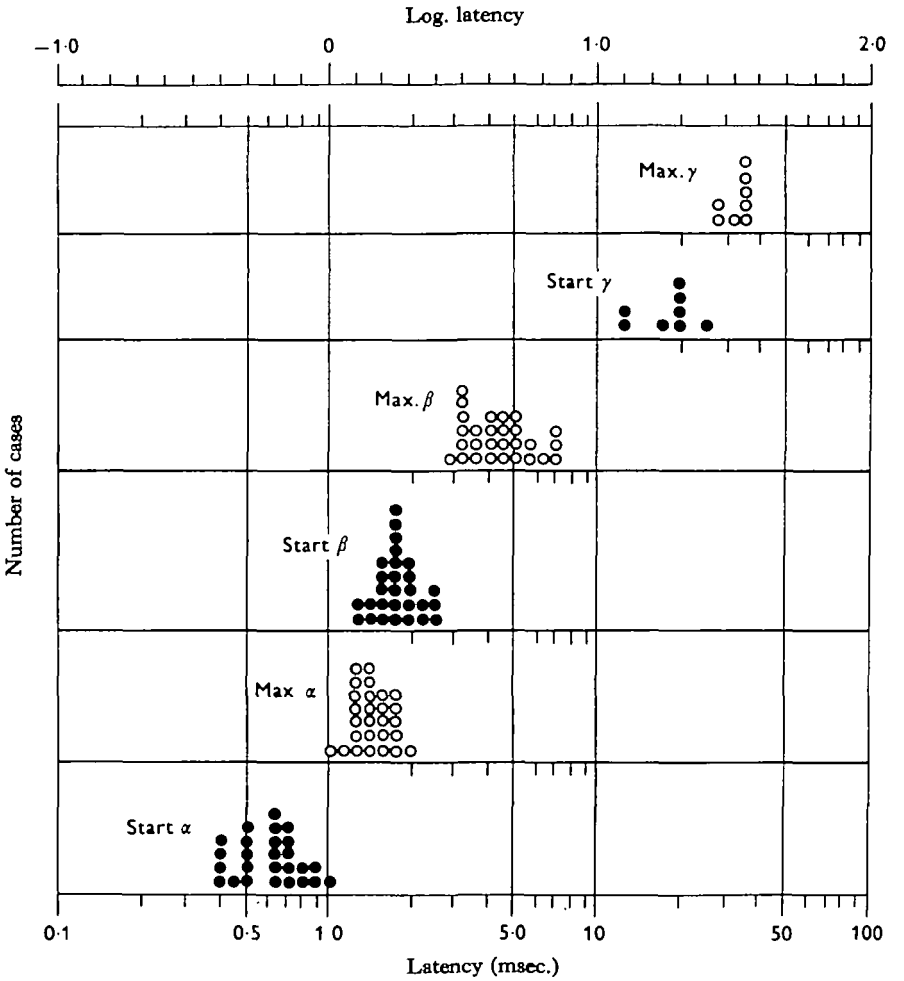

Fig. 5. Latency distributions of the start and maximum of each of the three components of the characteristic response in twenty-eight preparations. In all cases the second root stimulating electrodes were close to the ganglion and stimulus intensity was adjusted (roughly) to give a maximal subthreshold response. In a few cases measurements were not included on the histograms because they could not be made accurately from available records. There are relatively few measurements of the gamma component because in many experiments sweep speeds slow enough to include it were not used.

A. The early response

In intensity series such as those of Fig. 3, A_1 and B, the first response to make its appearance is usually a small depolarization which arises from the base line at about 0·5 msec. after the stimulus. We call this the alpha component of the total

response. It rises in about 0·8 msec. to a peak height which may be graded fairly finely by stimulus intensity variations (Fig. 3*B*) up to a maximum which varies from one preparation to another. The maximum is usually about 1·5 mV. but has ranged from being absent to being some 4 mV. in amplitude. Measurements on the alpha and later components are summarized in Fig. 5 and Table 1.

Table 1. *Means, standard deviations, and ranges for the amplitudes of the alpha, beta, and gamma components and the apparent critical level for spike initiation*

	Amplitude (mV.) of:			
Measure	Alpha	Beta	Gamma	Crit. level
Mean	1·6	7·0	4·7	7·65
Standard deviation	0·91	1·99	2·40	2·27
Range	0–4·0	5·2–13·6	2·2–6·8	5·5–16·0

We are confident that neither the alpha component nor those which follow it is either a stimulus artifact or an extracellular field potential from axons near the pipette tip for the following reasons:

(1) The alpha component can be seen (Fig. 4, C_1) to arise from a quiet base line well after the completion of the stimulus artifact and after the second root volley has begun to arrive at the ganglion. (2) If the second root is coagulated by passing a large, sustained current through the stimulating electrodes, all subsequent responses to second root shocks are abolished. (3) All responses are pure depolarizations. (4) All responses are abolished if the pipette tip is pushed or pulled barely out of the cell.

B. The later components

As stimulus intensity is increased slightly beyond the point where the alpha component usually appears, new elevations arise on its falling phase (Fig. 3, A_1, *a* and *b*, and *B*), and with further stimulus increases these appear to increase in number and coalesce to form a depolarization which continues to grow gradually in response to stimulus increases well after the amplitude of the alpha component has become asymptotic (Fig. 3, A_1–A_3, *f–h*).

Although this later depolarization ordinarily arises at the peak or early on the falling phase of the alpha component, in some preparations it appears in response to stimulation too weak to evoke the earlier response. Furthermore, a monopolar stimulating electrode tapered finely and insulated to near its tip can be placed so as to evoke the later response in absence of the earlier one, and conversely. Therefore, the afferent pathways for the alpha and later responses are at least partly separate.

As the later response continues to increase in amplitude with stimulus increments, its rate of rise also increases, and its contour becomes bimodal (Fig. 3*A*, *d–h*). We call the earlier, more rapidly rising and almost always larger mode the beta component and the later, broader hump the gamma component (Fig. 3, A_3, and Fig. 4). Once the beta component is fairly well developed as a monophasic peak, its median latency is about 1·8 msec. and its minimal latency 1·3 msec. (Fig. 5). It rises, often with some suggestion of 'treppe' (Fig. 3, A_1, and Fig. 11*A*), to a maximum which most

commonly measures some 6–8 mV. at about 4 msec. from a stimulus which has been set to evoke a maximal subthreshold response.

The falling phase of the beta component, and the gamma component as a whole, are particularly variable. Most commonly, if stimulus strength is fairly large, beta declines from its maximum rapidly at first and then abruptly slows its rate of descent (Fig. 3, A_1, *h*, and Fig. 4, B_2); gamma subsequently starts its rise at about 20 msec. and attains a broad maximum which averages 4·7 mV. at about 35 msec. when the stimulus is set to give a maximal subthreshold response. However, deviations from this situation are fairly frequent. Figure 4*E* illustrates a preparation in which there

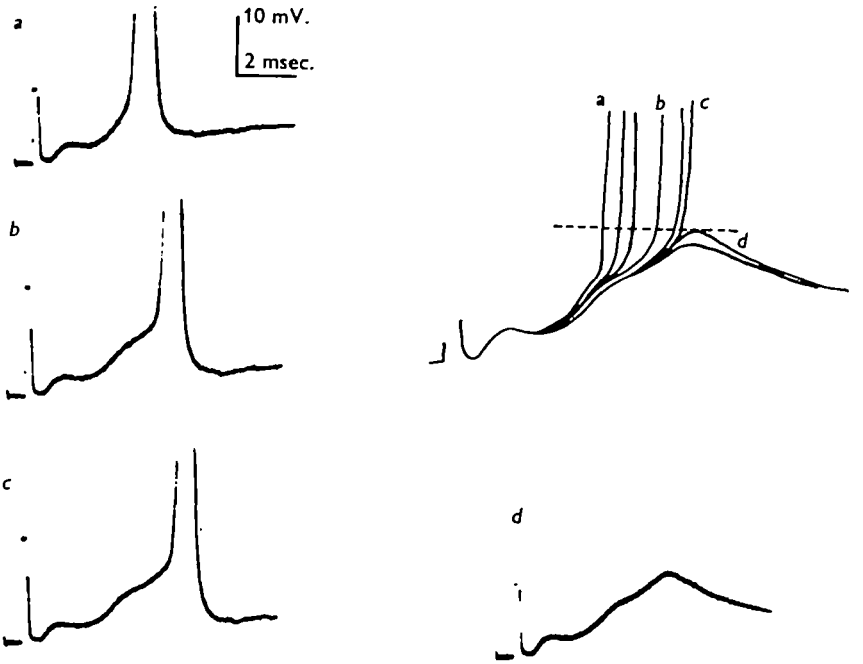

Fig. 6. Action potentials arising on the ascending limb of the beta component. In this experiment a constant second root stimulus which evoked a response sufficient to elicit a spike was repeated at a frequency which caused gradual reduction of the beta component. *a–d*, Selected sweeps from the series. Tracings of *a–d* together with several more sweeps of the series are superimposed on the upper right. – – –, The level of the largest beta component which did not evoke a spike.

is almost no fall after beta reaches its maximum and where gamma is larger than beta; Figure 4*D* illustrates an opposite extreme in another unusual preparation. After the gamma component has reached its maximum the depolarization declines gradually with no further elevations (Fig. 4, B_2). It returns to base line about 200 msec. after the stimulus which evoked it.

C. Spike initiation

Action potentials, which were usually 110–130 mV. in amplitude, could be elicited by second root stimulation in 81 % of those preparations tested with adequately large shocks. In all of these cases (21), the spikes arose from the ascending limb of the

beta component (e.g. Fig. 6). This occurred when it exceeded a critical depolarization which was roughly constant for a preparation and averaged 7·65 mV. (Table 1). However, the precise level at which an action potential develops depends rather strongly on the rise-time of the beta component as well as on its amplitude; the faster the rise, the lower the apparent critical firing level (Fig. 6).

The pattern of spike initiation shown in Fig. 6 gives the impression that it is the beta component that triggers the spike. The characteristics of the beta component can be varied either by alterations of stimulus intensity or by stimulus repetition (see below). Since the relationship between the beta component and spike initiation seems to be the same *however* a given size and rise-time for the beta component are arrived at, it is likely that the role of the beta component in spike initiation is indeed a causal one.

Finally, it should be mentioned that the depolarizing potential (Fig. 3, A_1, j) which follows action potentials is not the gamma component. It is rather, as has been shown by Roberts (1968), an inhibitory potential which is evoked by giant-fibre activity itself and serves to prevent the giants from firing for about 80 msec. following an initial spike. This post-spike potential will not be discussed further here. However, it at least partly explains why the lateral giants have never fired repetitively in any of the present experiments.

D. Other inputs to the system

A response with an early small and later large component whose initial portion can trigger spikes is also evoked by ipsilateral first-root stimulation. Furthermore, both an alpha and later components can be evoked by stimulation of either of the main branches of the second root. Therefore, the pattern of response which has been described is not peculiar to the particular place that was chosen for stimulation.

III. *Responses to repeated stimulation*

A. Response lability

The behaviour of the evoked depolarization has been examined at stimulus repetition rates ranging from 1/5 min. to 1/2·7 sec. in twenty-seven preparations though every preparation has not been tested over the entire range of frequencies. The alpha component is invariably stable at repetition rates well in excess of 2/sec. (though there is sometimes some small stimulus-independent drift). However, as we had expected from the behaviour of the intact animal, the later components are distinctly labile. The typical pattern of responses to repetitive stimulation is illustrated in Figs. 7 and 8.

In every preparation examined the beta component has suffered a decrement when tested at stimulus frequencies in excess of 2/min. (Table 2). There is commonly a marked decline over the first few trials and then a very slow one (Figs. 7, 10). Speed of decline depends on both stimulus strength and repetition rate, being faster for high repetition rates (Fig. 8) and weak stimuli (Fig. 10). In spiking preparations diminution of the beta response of course tends to cause an increase in spike latency leading to a cessation of firing (Figs. 8, 6).

Decrements at the lower frequencies used were also common (Table 2). However,

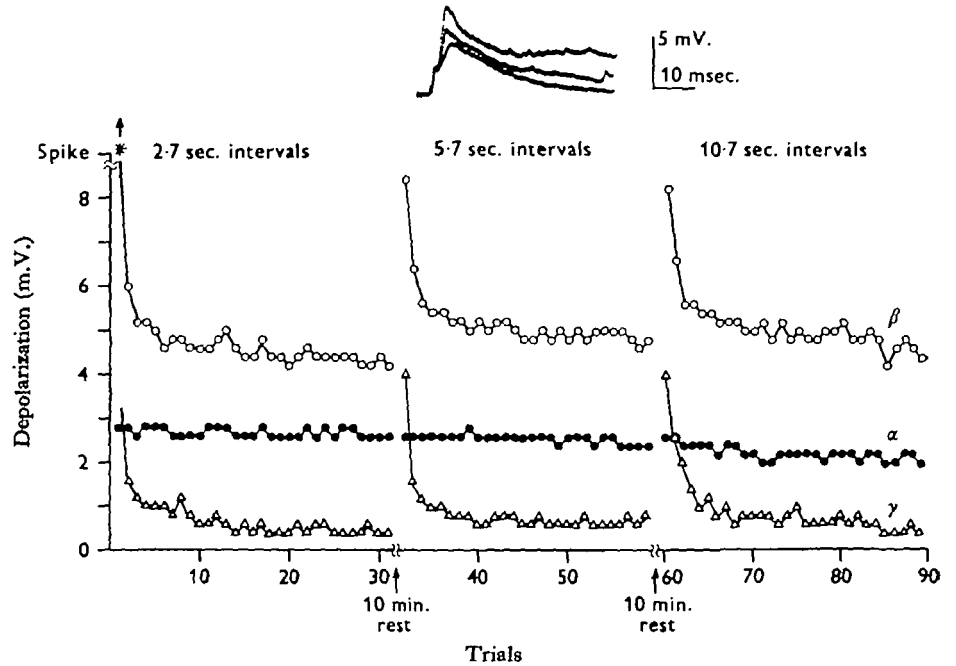

Fig. 7. The effects of stimulus repetition and rest. Three bouts of stimulation of fixed intensity at the repetition rates shown were separated by 10 min. rests. Inset above the middle panel are the first, second, and fifteenth responses from the series at 1 trial/5·7 sec. photographically superimposed.

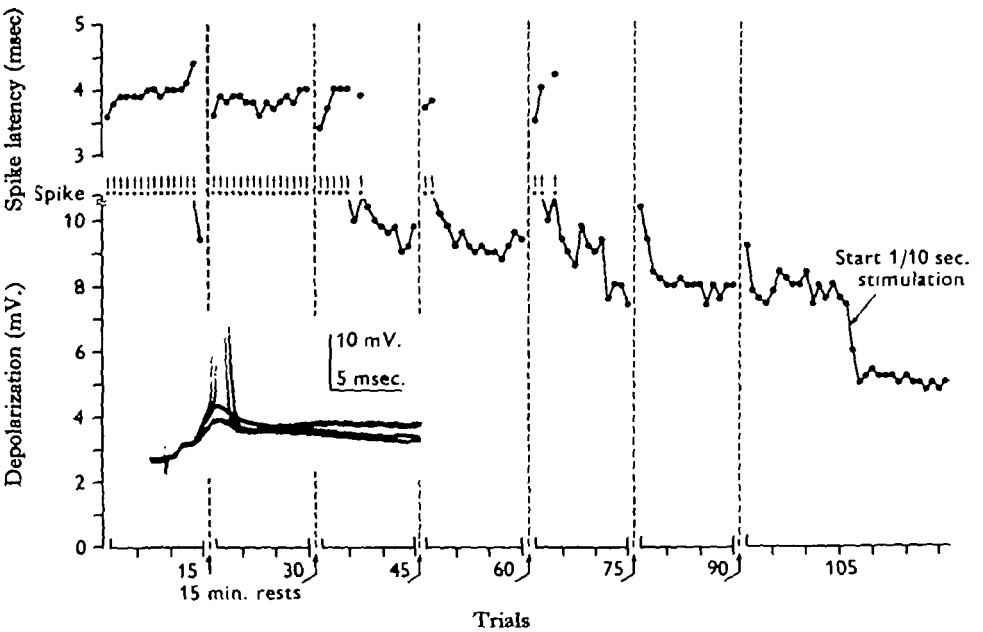

Fig. 8. Response failure as a result of stimulation once per minute. Blocks of 15 shocks at 1/min. were alternated with 15 min. periods of rest. The size of the beta component is plotted on trials without a spike; spike latency is plotted on trials with one. The oscilloscope traces from trials 61, 62, 63, and 75 have been photographically superimposed in the lower left of the figure.

occasionally in preparations stimulated only once every 5 min. the beta component actually *increased* during stimulus repetition.

The response alterations observed at frequencies of 1 stimulus/min. or faster cannot have been due to purely time-dependent changes in the condition of the preparations, since such alterations largely reversed themselves with rest (Figs. 7, 8). However, because of the limited life of these preparations it has not yet been possible to test for reversibility of changes occurring at longer interstimulus intervals.

Table 2. *The effect of repetitive stimulation on the beta component in 27 preparations*

Repetition rate ...	1/5 sec.*	1/10 sec.*	1/30 sec.*	1/min.	1/3 min.	1/5 min.
No. of preparations examined	12	7	4	8	5	13
No. showing decrease of response	12	7	4	6	4	8
Percentage showing decrease	100	100	100	75	80	62

* In some experiments the intervals were 0·7 sec. longer than stated in this table.

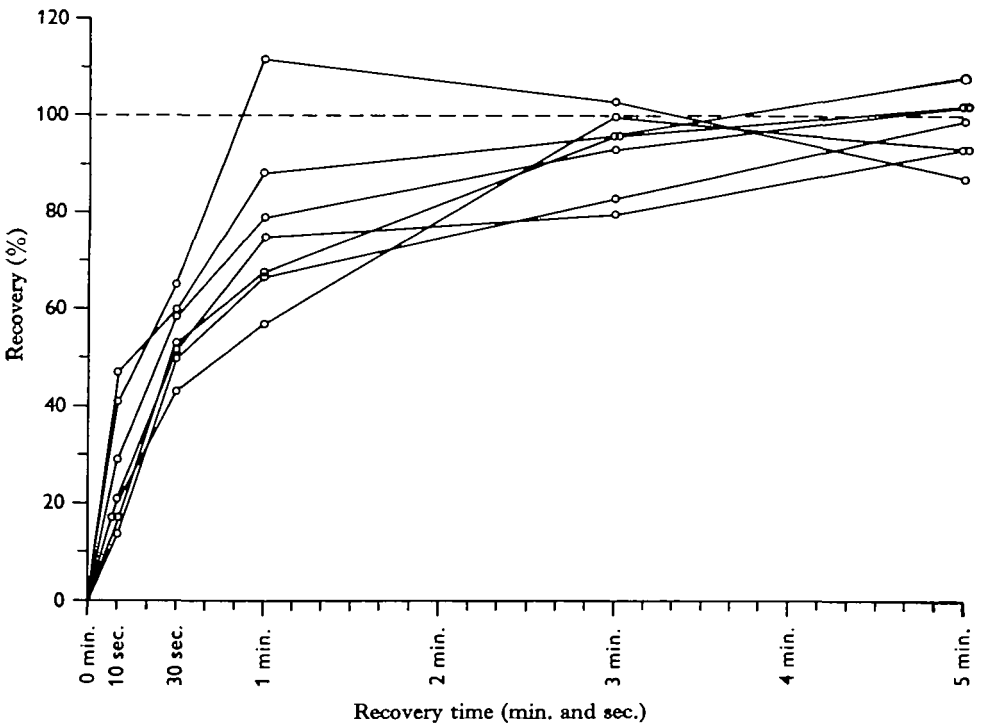

Fig. 9. Time course of recovery from reflex failure. Each point is the increment in beta component amplitude occurring during a rest of the stated duration and given as a percentage of the difference between the depolarization evoked on the first and last trials of the preceding conditioning bout. The lines connect the points from single animals.

In order to estimate the time-course of recovery from reflex failure, seven experiments were run in which a single test trial was given at various times after a conditioning bout of ten just-subthreshold stimuli at 1/5 sec. Test trials followed conditioning bouts by 10 sec., 30 sec., 1 min., or 3 min. Five minutes were allowed

to elapse between each test and the next conditioning bout. The initial trial of those conditioning bouts which followed 10 and 30 sec. test trials were used as tests of recovery during 5 min. of rest. Figure 9, which summarizes the results of these measurements, shows that the bulk of recovery occurs within 5 min.

The gamma component is commonly even more labile than the beta. In Fig. 7 (inset) it appears to be completely gone by trial 15, and often it shows a substantial decrease in size before beta changes at all. However, the extremely variable form of gamma both from trial to trial and preparation to preparation has caused us to focus our attention initially on the more robust, shorter latencied, and potentially spike-initiating beta component.

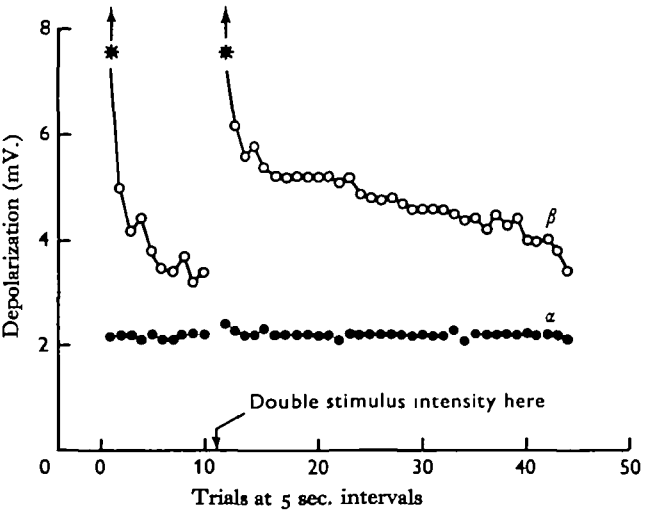

Fig. 10. The effect of doubling the stimulus intensity in an habituated preparation. Second root shocks generated by a constant current stimulator at 5 sec. intervals caused fairly rapid habituation during trials 1–10. Between trials 10 and 11, stimulus current was doubled. The beta component was transiently restored but declined again though less rapidly than before.

B. Stability of the afferent nerve response

In most of the above experiments the second root shocks evoking the response under study were submaximal; larger shocks would in most cases have increased the amplitude or stability of the beta component. Thus, there must generally have been a population of fibres in the second root which were being stimulated near their thresholds. The possibility that the decline of the beta and gamma components during repetitive stimulation might have been due to the dropping out of some part of such a population must be ruled out if the decrements described above are to be considered worth further analysis.

Decrements in the beta component can occur without obvious changes in the second root volley which is evoking the response. However, it is possible that very small changes in the afferent volley might have large effects on the evoked response; furthermore, second root fibres which are being stimulated near their thresholds do sometimes fail to fire reliably at stimulus repetition rates as low as 1/5 sec.

An alternative method of ruling out peripheral causes can be based upon the

fact that an individual fibre or small population, which is recorded in the second root with electrodes of 10–20 μ tip size, will always fire reliably (for at least 40 trials) at 2 sec. intervals if the stimulus voltage is set 33% above the rested fibre's threshold. Suppose then that decline of the beta component *were* primarily due to the dropping out of second root fibres. Then if the stimulus strength were increased 100% after beta had declined during a series of stimuli, any second root fibre which had dropped out during the series should resume and now maintain its response to second root shocks. As a consequence the beta potential should recover to better than its original size and should not fall below it. Figure 10 shows that when stimulus current is doubled there is a transient recovery as would be expected since new, higher threshold fibres are being made to fire, but the beta potential again declines to well below its original level. Therefore, failure of the beta component must involve central changes.

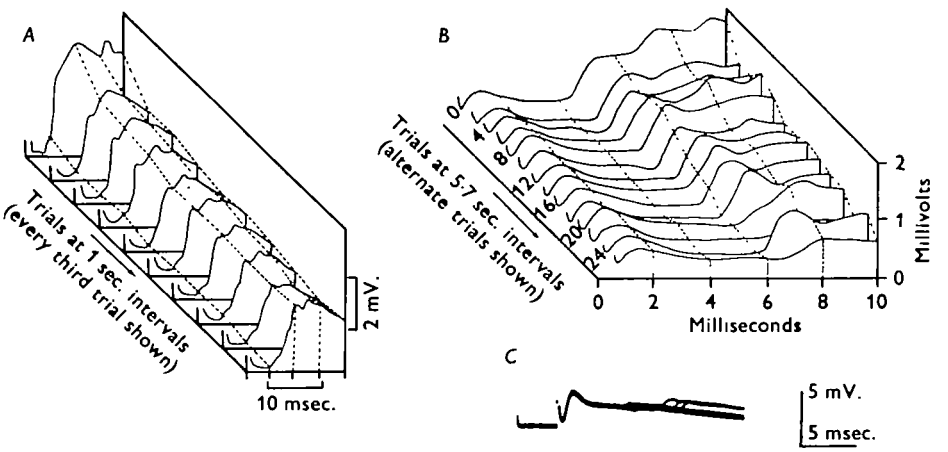

Fig. 11. The mode of failure of the beta component. In A and B, trials are ordered from back to front and the dashed lines are contours of equal time from the start of the stimulus. *A*, Trials were given at 1 sec. intervals to emphasize the composite character of the beta component; little or no alpha component is present. Notice that as the stimulus is repeated several new inflexions occur in the rising limb of the beta component whose maximum point also seems to shift in time. *B*, A fine, tapered wire insulated to near its tip was used to stimulate a small number of second root fibres at 5·7 sec. intervals. Notice that the subcomponent of beta thereby elicited (see text) increases in its latency by about 2 msec. before diminishing in size. (A and B traced.) *C*, Superimposed sweeps repeating at 2 sec. intervals. Notice the increase in latency and the all or none character of the variable component.

C. Further analysis of the decrement in the beta component

Despite its often uniform appearance, the beta component seems to be built up of a number of measurably large subcomponents. These are often revealed as inflexions in the potential's rising limb (Figs. 3, A_1, and Fig. 11*A*) or by the occurrence of several submaxima near its summit (Fig. 4, B_1, and Fig. 7). Inflexions which were not originally evident often appear during repetitive stimulation; one gets the impression that beta decreases in size partially as a result of increases in the latency and an accompanying cessation of synchrony of its subcomponents.

Since it is extremely difficult to maintain identification of a given subcomponent in a composite response, an attempt was made to obtain a few subcomponents of the

beta response in isolation by restricting the second root volley to a small number of fibres through the use of a stimulating electrode having a 30 μ tip. A depolarization was considered to be a single subcomponent if it was uniform in appearance and could not be graded in amplitude by variations in stimulus voltage. It was difficult to find examples which did not vanish after one appearance. However, Fig. 11*B* shows one of a number of successes which is typical in several regards. In the rested preparation (trial 0) the potential of interest rose from the tail of the alpha component a little more than 4 msec. after the stimulus and attained its maximum about a millisecond later. It was not feasible to obtain simple potentials at shorter latencies; if the stimulus voltage of this preparation had been increased, the beta component would have started earlier, but it would have been composed of a number of unresolved subcomponents. Although the latency to the start of this depolarization is rather long, the occurrence of its maximum at about 5 msec. after the stimulus characterizes it as a part of the beta component (see Fig. 5). The effect of stimulus repetition was to make the depolarization shift later in time while maintaining a constant amplitude until trial 20 when it seemed to fractionate into two parts; after that it was sporadic, failing on trial 22, recurring on trial 24, and so on. Ultimately, it failed entirely. The superimposed sweeps of Fig. 11*C* illustrate a similar pattern of failure particularly clearly in another preparation.

No clear case of gradual reduction in the size of a component either with or without change of latency has been found. Indeed, the decline of the normal beta component during repetition of large shocks is often a variable process; precipitous drops and sudden, transient reversals are common, especially at low repetition rates when the over-all course of decline is gradual. All these observations suggest that the waning of the beta component is due to the loss of synchrony and all-or-none failure of its subcomponents rather than to a gradual decrease in their size.

It is necessary, however, to caution reservation about this conclusion. First, direct data on the behaviour of individual subcomponents is available only for those which can be obtained conspicuously and in relative isolation at low stimulus intensities; these may not be typical. Secondly, although sudden failure of subcomponents preceded by increased latency at constant amplitude has been obtained with constant current stimulation at frequencies as low as 2/min., we cannot categorically rule out the possibility that changes in the second root volley are involved.

DISCUSSION

The evoked response

Most of the experiments reported here have utilized single-shock stimuli. Though the responses evoked by such stimuli are of course artificial, it has been useful to start analysis of the escape reflex in this way because it has allowed the separation by differences in latency of several reflex pathways differing in their physiological properties.

The very short latency of the alpha component and its stability during stimulus repetition suggest that it is probably a monosynaptic response. However, its small size has made us wonder whether it might be an artifact of synchronous antidromic activation of second (and first) root efferents. If this were so, some extensor excitors

would have to be involved to account for the observed degree of gradation of the component (Fig. 3*B*), and this seems unlikely since direct synapsis between lateral giants and extensor excitors would then presumably have to be assumed. Therefore, the alpha component is probably not an artifact, but its significance does remain obscure.

By contrast the relatively large size and spike-initiating potentiality of the beta component leave little doubt as to its functional importance. Its minimum latency of 1·3 msec. allows time for traversal of two (or at most three, given some error in latency measurements) chemical synapses, and its behaviour during stimulus-strength variations and stimulus repetition are what would be expected of an EPSP arriving over a disynaptic pathway with convergence at each synapse and marked lability at the first. However, Takeda & Kennedy (1965) have presented evidence from other interneurones of crayfish abdominal ganglia showing that depolarizing responses which look and behave like EPSPs are in fact often the summed electrotonic effects of spike potentials localized in many individual axon branches at some distance from recording electrodes. Since recording pipettes in the body of the lateral giants do not seem particularly well placed for picking up EPSPs generated distally in the axonal tree, it seems distinctly possible that the beta component is predominantly made up of such distant spikes. If this is so, then its 1·8 msec. (median) latency must include, in addition to afferent conduction time and synaptic delays, time also for unseen EPSPs to rise to a level where they can initiate branch spikes and perhaps time for these in turn to propagate actively in the fine fibres where they presumably arise. Thus it remains possible that the beta component, despite the indications of its latency and other properties, is monosynaptic.

The possible interpretations of the long-latencied gamma component are several. It could equally well represent (1) input over a polysynaptic interneuronal pathway, (2) the asynchronous arrival of second and later spikes from a number of repetitive spike trains whose first impulses arriving synchronously evoked the beta component, (3) electrotonus from the EPSPs which generated the branch spikes whose summed effect may in turn have constituted the beta component, or (4) effects of summed, asynchronous branch spikes generated, after recovery of the branches from refractoriness, by the later portions of the same unseen EPSP that produced the beta component. Experiments designed to select between these alternatives and those of the preceding paragraphs are in progress.

Lability of the beta component

The behaviour of the beta component during stimulus repetition closely resembles that seen in several other recent investigations with goals similar to those of this one. Thus, Bruner & Tauc (1966) have reported decreases of EPSP size in the left pleural ganglion giant cell of *Aplysia* during 1/10 sec. shocks to cerebral nerves or connectives; Spencer, Thompson & Neilson (1966*a*) report decreases in flexion–reflex responses of acute spinal cats during 1–3/sec. single shocks or 1/10 sec. bursts of shock to skin or cutaneous nerves; and Wickelgren (1967*a*, *b*) reports decreased responding in both motor neurones and dorsal horn interneurones during 1/10 sec. bursts of shocks to cutaneous or high-threshold muscle afferents. At least in the case of the spinal flexion reflex, response diminution did not occur if rests of 1 min. between single shocks or 1–4 min. between bursts were allowed. Recovery time in

all cases ranged between 30 sec. and 30 min. The lability of the beta component has similar temporal characteristics.

At issue in these investigations has been the question of whether response waning is due to a decrease in the reactive properties of some part of the reflexes' excitatory pathways or to extrinsic gating by means of presynaptic inhibition, postsynaptic inhibition, or a decrease in tonic facilitatory bombardment. In the case of *Aplysia*, EPSP amplitude has been seen to decrease gradually without any postsynaptic sign of facilitatory or inhibitory modulation; presynaptic effects could not, however, be dismissed. In the spinal cord the only relevant evidence comes from pharmacological experiments which tend to rule out inhibitory gating (Spencer, Thompson & Neilson, 1966*b*).

We are similarly inclined to dismiss inhibitory gating in the present preparation on the basis of experiments with picrotoxin (Krasne & Roberts, 1967). It is additionally true that there is no sign of modulation by facilitatory or inhibitory events; however, we would expect to see such signs only if we were recording near a place where reflex transmission actually becomes blocked, whereas the saltatory mode of failure of unitary contributions to the beta component suggests that blockage in fact occurs at a site distinctly afferent to our recording electrodes.

Saltatory failure could result from blockage at (1) synapses on to neurons intercalated between afferents and giant fibres, (2) synapses on to electrotonically remote parts of the giant fibre's axonal arborization (assuming mediation by branch spikes), or (3) points of low safety factor for spike propagation in the arborization. In the last case it is particularly possible that blockage might result from waning of electrical excitability in fine axon branches; however, if this were so, one would have to assume that antidromic spikes do not invade the axonal tree, since direct stimulation of giant fibres does not seem to affect their reflex excitability.

Relation to behaviour

Habituation of the escape reflex in the intact animal, which is what we have set out to understand, occurs within a few trials when natural stimuli are delivered at 5 min. intervals and recovers only during a number of hours of rest. In contrast, when afferent nerve shocks which evoke depolarizations near threshold for spike initiation are repeated at 5 min. intervals, the beta component declines either very slowly or not at all, and the substantial decrements which can be produced by more frequent stimulation largely dissipate in minutes rather than hours. Thus it does not appear that the lability of the beta component in the isolated tail can explain the lability of the escape response in the intact animal. In consequence we must entertain as possibilities that (1) responses to afferent nerve shocks are much less labile than responses to natural stimuli, (2) contrary to what is commonly believed from the work of Wiersma (1947), the escape response of the intact animal is not ordinarily mediated by the giant fibres, (3) the expression of strong lability in transmission between segmental afferents and lateral giants depends upon the influence of the rostral portions of the nervous system which were removed in these experiments, or (4) long-lasting depression could not be established during testing because it was already complete before testing was begun. All of these possibilities are under investigation; work in progress suggests that the third may be correct.

In conclusion we should like to emphasize that if one wants to discover how nervous systems *actually* bring about behavioural plasticity rather than how they *might* do so, then there are distinct advantages in starting with a labile behaviour pattern and proceeding to its analysis rather than starting with a plastic physiological process and hoping that it will turn out to be used by the nervous system in the modification of behaviour. This is why we have chosen the crayfish escape response for analysis.

SUMMARY

The tail-flip escape reflex of the crayfish shows marked habituation and is very amenable to detailed electrophysiological study. It thus provides a model system for comprehending the neural basis for one sort of learning phenomenon. This paper describes the electrical events which can be recorded by micropipettes placed in lateral giant axons of isolated crayfish abdomens.

1. There is little spontaneous activity. Depolarizing responses can be evoked in a given segment by natural or electrical stimulation ipsilateral or contralateral to the recording electrode and at any of a number of segments.

2. Electrical shocks to the ipsilateral second root of the impaled ganglion evoke a depolarization with an early stable phase and a later labile phase which shows largely reversible decrements when stimulated as seldom as once a minute. Only the labile portion is ever big enough to trigger a propagated response.

3. The initial, potentially spike initiating, and largest portion of the labile phase has a sufficiently short latency to suggest that it arrives along a disynaptic or at most trisynaptic pathway.

4. The labile phase appears to be built up of a number of measurably large, unitary, subcomponents. Decrements during repetitive stimulation seem to result from the all-or-none dropping out of these subcomponents following increases in their latency. This suggests that blockage of transmission occurs at synapses on to neurones intercalated between afferents and giant fibres or at parts of the axonal tree which are electrotonically remote and support branch spikes.

5. The lability described here is probably not sufficient to account for habituation in the intact animal.

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