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PROTECTION FROM HABITUATION OF THE CRAYFISH LATERAL GIANT FIBRE ESCAPE RESPONSE

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SUMMARY

1. Habituation of the lateral giant fibre escape response in the crayfish to repetitive tactile stimuli is believed to result from homosynaptic depression at the first synapse of the reflex, between tactile afferents and interneurones. Normally, habituation of escape responses to repeated innocuous stimuli is presumed to be adaptive. Experiments reported here were undertaken to determine whether habituation would occur under circumstances when it would presumably be maladaptive – in particular, when tactile receptors are stimulated by an animal's own tail-flip movements.

2. Experiments were carried out on the crayfish isolated abdominal nerve cord, which contains the lateral giant reflex pathway.

3. Compound e.p.s.p.s elicited in the lateral giant by electrical stimulation of tactile afferents decline by from 25 to 36 % over a series of eleven trials at 1/5 sec (control series).

4. To determine whether such a decline would occur when sensory afferents are stimulated during a 'tail-flip', stimuli were given as in the control series but each stimulus occurred 20 msec after direct electrical stimulation of a medial giant or lateral giant escape-command fibre at which time tail flexion movements of an intact animal would be in progress. Under these conditions % e.p.s.p. decline over 11 trials at 1/5 sec was only 16–45 % of that occurring on the control series.

5. This protective effect starts at about 10 msec after escape command neurone firing, is maximal at 20 msec, and thereafter declines, remaining weakly detectable at 100 msec. This time course is commensurate with that required for execution of a tail-flip movement. Thus, sensory afferent-to-lateral giant transmission is protected from depression if stimuli occur when a tail-flip movement is or should be occurring.

6. Giant fibre spikes do not superimpose facilitation upon a depressed reflex pathway, nor accelerate rate of recovery from depression; rather,

protection is attributable to actual prevention of development of the depressed state.

7. Protection was also examined at the first synapse of the reflex, where the depression responsible for habituation is believed to occur, by recording intracellularly in the largest of the first-order interneurones (interneurone A) of the pathway. In absence of protection, ten stimuli presented at 1/4 sec caused a mean decline of 32 % in the e.p.s.p. in interneurone A. When such stimuli followed directly evoked escape command neurone firing by 20 msec this decline was reduced by 59–100 %.

8. We suggest that protection serves to prevent crayfish from habituating to stimuli produced by their own tail-flip movements.

INTRODUCTION

Many synapses exhibit altered efficacy of transmission upon repeated activation of the presynaptic element, with the consequence that their functional properties may be significantly modified by their previous history of activity. In some instances where considerable information exists concerning the functional roles of particular synapses it has been possible to ascribe adaptive consequences to these synaptic alterations. Decreases in synaptic efficacy, for example, are responsible for habituation of a number of escape and withdrawal responses (e.g. Castellucci, Pinsker, Kupfermann & Kandel, 1970; Krasne, 1969; Roberts, 1968; Zilber-Gachelin & Chartier, 1973*a*,*b*; Zucker, 1972*a*,*b*). Such habituation is generally considered (e.g. Thorpe, 1956) to benefit the organism by reducing metabolically costly responses to insignificant stimuli and by permitting non-escape activities such as feeding to occur in turbulent but safe environments. Increases in synaptic efficacy, such as those which occur prominently at many arthropod neuromuscular junctions (e.g. Dudel & Kuffler, 1961*b*; Kennedy & Takeda, 1965; Sherman & Atwood, 1971; Wiersma, 1970), are thought to be used to increase the range and complexity of effector responses producible by neuromuscular systems with few motor units (e.g. Atwood, 1973; Wilson & Davis, 1965). We presume that, as is the case in these examples, most synapses whose efficacy tends to alter with use are so constituted for functionally meaningful reasons. However, in the few cases where the biological value of synaptic plasticity can be seen, one can also envision special conditions in which the alterations would be maladaptive. Under these conditions it would be advantageous if the tendency to alter could be suppressed.

Although Wiersma & Yamaguchi (1967) report in passing that detectors or visual 'jittery movements' in crayfish optic tract tend not to habituate when an animal moves its *own* eye stalk, little direct attention has been

given to the possibility that neuronal plasticity might be modulatable. We thus undertook experiments to evaluate this possibility by asking whether alterations involved in producing habituation of the lateral giant escape reflex of the crayfish occur under circumstances where habituation should be maladaptive.

The lateral giant escape reflex is a rapid abdominal flexion (see Wiersma, 1947) that occurs in response to sudden tactile stimulation of the crayfish abdomen (Wine & Krasne, 1972), propelling the animal up and back through the water (Larimer, Eggleston, Masukawa & Kennedy, 1971; Wine & Krasne, 1972). When a particular spot on the abdomen is stimulated repeatedly the probability of response greatly diminishes (Wine, Krasne & Chen, 1975); this habituation is believed to be due to decreased efficacy of transmission (homosynaptic depression) at the first synapse of the reflex pathway (Zucker, 1972*a*,*b*; Krasne, 1969; Krasne & Bryan, 1973). Since the animal's own tail-flips would be expected to activate the abdominal tactile receptors, which are very sensitive to water currents, tail-flip movements should lead to habituation of escape behaviour with the result that tail-flips serving ends unrelated to escape would leave the animal less likely to escape from subsequent tactile threats. Therefore, it should be adaptive for the initial labile synapses of the escape reflex to be protected from becoming depressed during tail-flips (or other vigorous movements). The experiments presented here provide evidence of such protection. A preliminary account has appeared elsewhere (Krasne & Bryan, 1973).

METHODS

Review of the lateral giant escape reflex

Relevant aspects of the known circuitry of the lateral giant reflex are diagrammed in Fig. 1 (Zucker, Kennedy & Selverston, 1971; Zucker, 1972*a*). Tactile afferents drive a single tier of interneurones, three of which (A, B, and C) are identified cells (Wiersma & Hughes, 1961; Kennedy, 1971). These converge on the *lateral giant* command neurone, which in turn drives a large population of motor neurones (Furshpan & Potter, 1959; Takeda & Kennedy, 1964; Selverston & Remler, 1972; Mittenthal & Wine, 1973) whose concerted action is to rapidly flex the abdomen. Other neurones, including the *medial giants* and various unidentified non-giant command neurones for tail-flip responses, also have access to this motor neurone population (see Wiersma, 1947; Atwood & Wiersma, 1967; Schrameck, 1970; Wine & Krasne, 1972; Bowerman & Larimer, 1974).

Synaptic transmission from tactile afferents to interneurones, which is believed to be chemically mediated (Kennedy, 1971; Zucker, 1972*a*), is labile; this lability is thought to be responsible for habituation (Zucker, 1972*b*). The other central excitatory synapses in the system are electrical and appear to transmit stably (Zucker, 1972*a*,*c*).

Experimental procedures

All experiments were performed on *Procambarus clarkii* 6·5–8 cm in length from rostrum to uropods. Before dissection, the crayfish were cooled gradually to 5° C in a dish of dechlorinated tap water. Surgery was in 8° C van Harreveld's solution (van Harreveld, 1936). Following surgery the nerve cord was continuously perfused with heavily aerated van Harreveld's solution at about 17° C. Experiments lasted 3–5 h from the completion of dissection. In all experiments unnecessary stimulation was avoided and habituation series were separated from one another by at least 5 min to permit recovery. For additional details see Krasne, 1969.

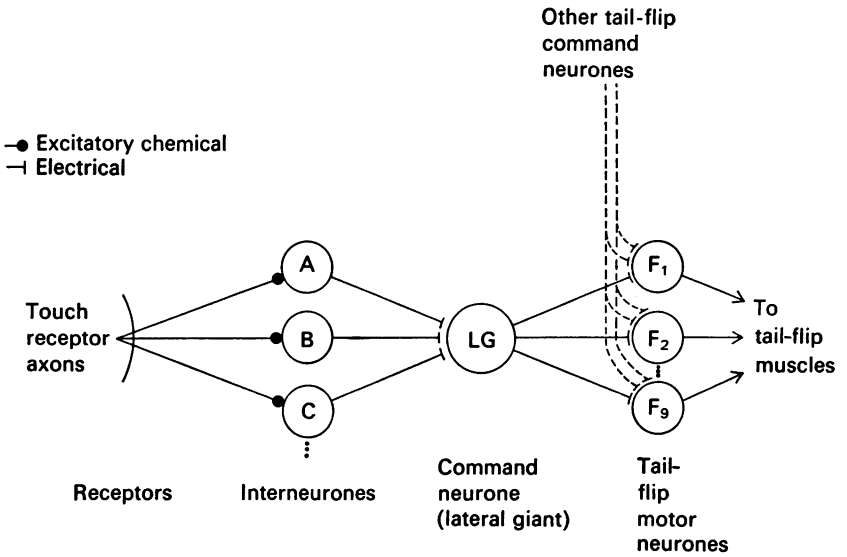

Fig. 1. Schematic diagram of the lateral giant escape reflex circuit of the crayfish (based largely on Zucker, Kennedy & Selverston, 1971; Zucker, 1972*a*). See text. Several known features of the reflex that do not bear on the present work are omitted.

Procedures for experiments at the command neurone level. The isolated abdomen was pinned dorsal surface up in a dish filled with cold van Harreveld's solution (van Harreveld, 1936). The ventral nerve cord was exposed and the motor roots to flexor musculature cut (details as in Krasne, 1969).

Lateral giant fibres were impaled with glass micropipettes (2·5 M-KCl-filled; 5–10 MΩ resistances) just rostral to the septal synapse in third or fourth abdominal ganglia. Measured resting potentials ranged from 80 to 90 mV. Action potentials were over-shooting and 100–120 mV in amplitude. Sensory afferents to the impaled lateral giant were stimulated electrically through a bipolar platinum hook electrode placed under the ipsilateral second root close to the ganglion in the same abdominal segment.

Selective stimulation of a single medial giant fibre was via an electrolytically sharpened insect pin, insulated to near the tip (exposed tip diameter *ca.* 50 μm), placed on the dorsal surface of an abdominal connective near the mid line. In a few

experiments, focal stimulation of a lateral giant fibre was accomplished by impaling the lateral giant axon in a connective other than that containing the recording micro-electrode. Giant fibre spikes were monitored by a gross recording electrode on the dorsal surface of the nerve cord.

Intracellular potentials were amplified by Medistor or WPI electrometer amplifiers; extracellular potentials were amplified by Tektronix 122 preamplifiers. Output from the preamplifiers was displayed on an oscilloscope and photographed conventionally.

Procedures for experiments carried out at the first synapse. Mechanical instability of the abdominal nerve cord *in situ* made stable intracellular penetrations of the first-order interneurone (interneurone A) extremely difficult. Therefore, the abdominal nerve cord was removed from the abdomen, and pinned ventral side up on Sylgard.

Interneurone A was identified by (1) the large size of its action potential at the ventral surface of an abdominal connective, (2) its short (typically 2–4 msec) latency in the 5/6 abdominal connective to orthodromic stimulation, (3) its low threshold for activation by repetitive electrical shocks to any of ipsilateral roots 1 to 4 of the last abdominal ganglion, and (4) its ventrolateral position in the last abdominal connective.

The nerve cord was positioned so that the dorsal surface of a rostral connective lay across a bipolar silver stimulating electrode used for activating the cord giant fibres.

Stimulation and recording were generally as above; however, finer micro-electrodes (15–30 mΩ resistances) were used for penetration of interneurone A at exit of its axon from the 6th abdominal ganglion. Intracellularly recorded action potentials in this cell were 95–110 mV in amplitude, and resting membrane potentials ranged from 75 to 80 mV.

RESULTS

Intracellular recording from the lateral giant fibre

Reduction in the amplitude of compound e.p.s.p.s in lateral giant to repeated activation of tactile afferents. The synaptic changes in the lateral giant escape reflex pathway that underlie short-term habituation occur afferent to the lateral giant at the first synapse of the reflex (Fig. 1; see Zucker, 1972*a*,*b*; Zucker *et al.* 1971) and result in a progressive decline in the amplitude of compound e.p.s.p.s recorded intracellularly in the lateral giant when electrical stimulation of reflex afferents is repeated (Krasne, 1969). This decline in e.p.s.p. amplitude is shown in Fig. 2*A* for trials 1, 2, 3, and 11 of a series of second root stimuli given at 1/5 sec. The stimulus intensity was adjusted to be just below threshold for an action potential in the lateral giant on trial 1. Over 11 trials, the peak amplitude of the e.p.s.p. decreased by 33%. This decline of compound e.p.s.p. amplitude serves in an acute preparation as a neuronal analog of behavioural habituation in the intact animal. The mean percent decline of e.p.s.p. amplitude in six preparations during stimulus series identical to that in Fig. 2*A* is summarized in Table 1 (column II). The percentage decline was calculated as $[1-(\text{e.p.s.p. amplitude Trial 11/e.p.s.p. amplitude Trial 1})]\times 100$. During eleven trials at 1/5 sec the average decrease in the peak amplitude

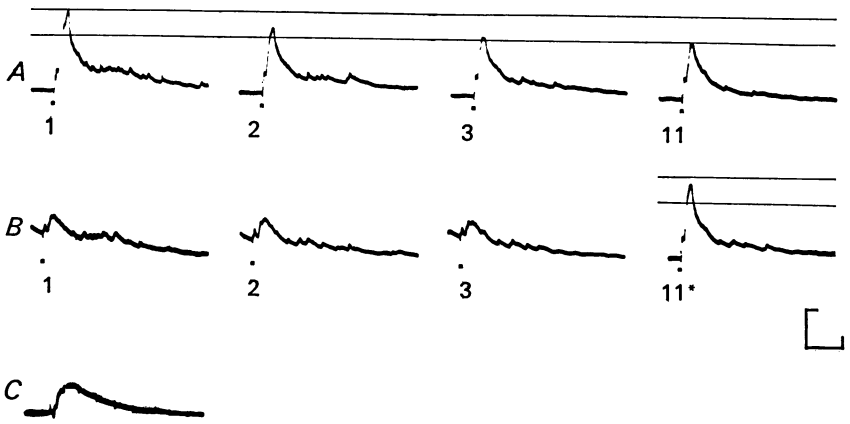

Fig. 2. Compound e.p.s.p.s in the right LG of the 3rd abdominal ganglion evoked by brief shocks to the ipsilateral second root.
A, eleven second root shocks (square markers) were delivered at 1/5 sec (trials 1, 2, 3 and 11 are shown).
B, eleven second root shocks identical to those in 2*A* were given; however, 20 msec before each of the first 10 shocks a single suprathreshold focal electrical stimulus to a medial giant in the 4/5 abdominal connective was given (medial giant stimuli occurred prior to triggering the oscilloscope sweep).
C, depolarizing i.p.s.p. in the lateral giant fibre to focal stimulation of a medial giant. Elevation of the base line at the start of sweeps in *B* 1–3 is due to this i.p.s.p. *A*, *B*, and *C* are from the same preparation. Calibration: 4 mV and 20 msec.

TABLE 1. Declines in compound e.p.s.p.s recorded from the lateral giant fibre to second root stimuli alone or in combination with medial giant stimuli

I	II	III								
	Mean	Mean Experimental decline (as % of mean control declines)								
	control	Medial giant/second root interstimulus interval (msec)								
Experiment	decline	−20	−10	0	10	20	30	50	100	150
J9	27·7 ($n = 4$)	—	—	—	—	37·3 ($n = 4$)	52·2 ($n = 1$)	94·5 ($n = 3$)	92·3 ($n = 2$)	—
J11	36·5 ($n = 5$)	—	—	—	86·5 ($n = 2$)	45·3 ($n = 5$)	36·9 ($n = 2$)	76·3 ($n = 2$)	87·6 ($n = 2$)	—
J13	32·2 ($n = 4$)	—	—	—	60·9 ($n = 3$)	37·1 ($n = 3$)	—	28·3 ($n = 3$)	88·4 ($n = 2$)	86·3 ($n = 2$)
J15	29·5 ($n = 4$)	99·4 ($n = 2$)	90·9 ($n = 2$)	105·0 ($n = 3$)	36·5 ($n = 2$)	37·9 ($n = 2$)	—	—	—	—
J16	25·2 ($n = 5$)	—	99·9 ($n = 2$)	99·0 ($n = 3$)	—	16·8 ($n = 1$)	—	—	—	—
J17	25·7 ($n = 5$)	—	124·9 ($n = 4$)	117·0 ($n = 4$)	—	—	—	—	—	—

of the compound e.p.s.p. in the lateral giant ranged from 25·2% to 36·5% across preparations.

'Protection' from habituation during 'tail-flips'. If the nervous system of the crayfish possesses a mechanism which prevents behavioural habituation of the lateral giant reflex to stimuli generated by the animal's *own* tail-flips, it should be possible to demonstrate that such a mechanism reduces or prevents the decline in amplitude of compound e.p.s.p.s shown above. In order to simulate the patterns of central neuronal activity occurring during the course of a tail-flip, a medial giant escape command fibre (a lateral giant could equally well have been used) was fired by direct shocks (see Methods and Fig. 1). Afferents to the lateral giant were then stimulated following the medial giant spike at a time when an intact animal would have been executing a tail-flip (Roberts, 1969; Wine & Krasne, 1972).

A series of ten such stimulus pairs, medial giant followed 20 msec later by a shock to second root afferents, was given (Fig. 2*B*), and on the last trial of the series (trial 11*) the afferent stimulus was given alone to determine the amount of decline in e.p.s.p. amplitude that had resulted from the previous ten afferent stimuli (identical to and from the same preparation as those in Fig. 2*A*). Comparison of trial 11 of the control series and trial 11* of the experimental series shows that less decline occurred when each afferent stimulus was preceded by medial giant firing.

The percentage decline occurring on experimental series could not be determined by directly comparing trials 1 and 11* of the series because during trials 1-10 e.p.s.p.s were markedly attenuated by post-synaptic inhibition of the lateral giant which follows medial giant (or lateral giant) firing (see Fig. 2*B*, Roberts, 1968, and the following paper). Therefore, control series were given frequently throughout an experiment, and 'e.p.s.p. amplitude trial 1' in the formula for percentage decline (see above) was taken as the mean of the e.p.s.p. amplitudes on the initial trials of the control series that most closely preceded and followed the protection series in question. Computed in this way, percent decline over the experimental series of Fig. 2 was 11% as compared to 33% for the control series. The experimental decline was therefore 33% of the control series decline. Comparable experiments on 5 preparations gave experimental declines that were from 16·8 to 45·3% of corresponding control declines (Table 1, column III, 20 msec interstimulus interval).

Percent protection defined as (1 − % decline on experimental series/% decline on control series) × 100, thus ranged from 54·7 to 83·2%.

Protection also results from lateral giant firing. Fig. 3 shows the response of the right lateral giant impaled just rostral to the septal synapse in the third abdominal ganglion to the first, second, and last of eleven second

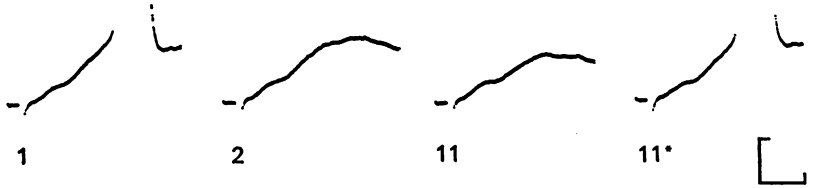

Fig. 3. Intracellular responses of the right lateral giant impaled just rostral to the third abdominal ganglion. Traces 1, 2 and 11 are responses to the first, second and eleventh 2nd root shock given at 1/5 sec. Response 11* is from an identical series of second root stimuli except that each of the first 10 shocks (not shown) was preceded at 20 msec by a single supra-threshold stimulus delivered to the right lateral giant in the last abdominal connective via an intracellular micro-electrode. Calibration: 4 mV, and 2 msec.

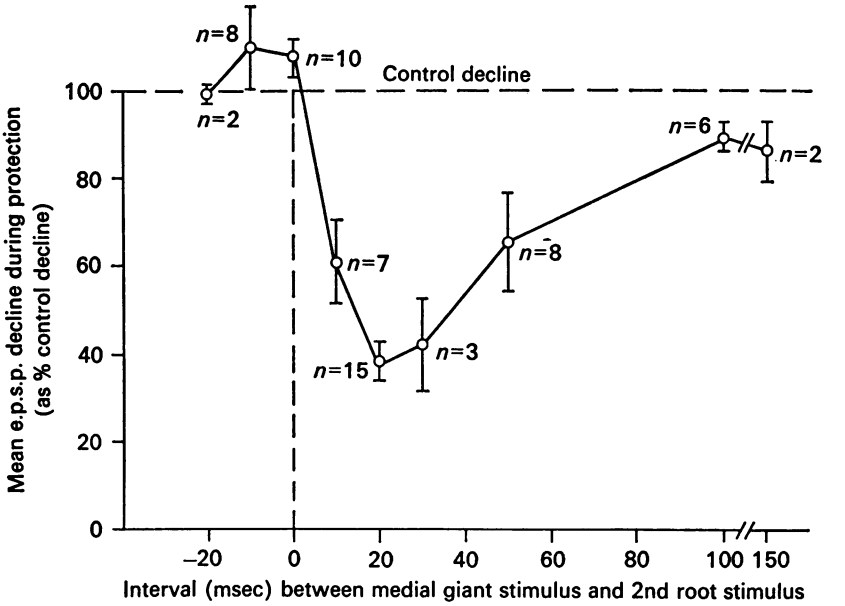

Fig. 4. Mean % e.p.s.p. decline in the lateral giant fibre produced by repetitive second root stimuli presented while the preparation was 'protected', expressed as the percent of the mean decline occurring on adjacent control runs. Protection series consisted of 10 second root stimuli at 1/5 sec with each second root stimulus following (positive intervals on the abscissa) or preceding (negative intervals) a single medial giant stimulus. On control runs second root stimuli were presented alone at 1/5 sec. Data from all runs at a given interval were pooled. Not all intervals were tested in each experiment (see Table 1). Error markers are S.E. of the mean of the pooled data at each interval.

root shocks given at 1/5 sec. The second root stimulus intensity was adjusted to be just suprathreshold for an action potential in the lateral giant on trial 1. On trials 2 to 11 the declining excitatory input failed to depolarize the lateral giant to its critical firing level. However, when each of the first 10 second root stimuli was preceeded at 20 msec by a suprathreshold intracellular stimulus to the right lateral giant impaled in the last abdominal connective, the e.p.s.p. decrement over the conditioning trials was greatly reduced, so that on the 'test' trials (trial 11*) the second root stimulus given alone resulted in an action potential in the lateral giant.

Time course of protection. The same paradigm was used to determine the time course of the protection effect. Focal extracellular stimulation of a medial giant was followed (or preceded) at different interstimulus intervals by a second root stimulus. Ten stimulus pairs were presented at 1 pair/5 sec while the interval between medial giant and second root stimuli was varied between series (Table 1, Fig. 4). The onset of protection occurs somewhere between 0 and 10 msec after the medial giant stimulus. It reaches peak effectiveness at roughly 20 msec (at which time mean e.p.s.p. decline is only 38·6 % of that occurring on control (unprotected) runs), and appears to persist for over 100 msec following a medial giant spike. Since giant fibre-mediated tail-flips begin approximately 8–10 msec after stimulus delivery and require about 100 msec for a complete cycle of flexion and extension (e.g. Roberts, 1968), the time course of protection determined in our experiments corresponds reasonably well to that of giant fibre-mediated tail-flips. The overall time course of protection is therefore consistent with the function we have postulated for it.

Lack of effects of giant fibre activity per se on excitability in the lateral giant pathway. Several observations support the hypothesis that the effect of a preceding giant fibre spike is really to 'protect' the lateral giant reflex pathway from habituation, rather than to superimpose a facilitatory effect ('sensitization') upon ongoing decremental synaptic changes or perhaps to cause true dishabituation.

On protection series in which giant fibre spikes *follow* each second root stimulus (see Table 1 and Fig. 4, negative intervals) the ten giant fibre spikes occurring during the conditioning trials do not result in reduced decrement of e.p.s.p.s compared to the decrement occurring during control runs. In a separate test for sensitization, ten suprathreshold giant fibre shocks were delivered at 1/5 sec (providing the same number and timing of giant fibre spikes as occurred in the protection conditions), and 5 sec after the last such shock, a second root stimulus was given. The amplitude of the response to this second root stimulus which followed giant fibre stimulation was no greater than that produced by a single second root stimulus presented to a rested preparation.

The possibility that giant fibre spiking accelerates *recovery* from reduced reflex excitability was also examined in two preparations. Fig. 5 presents data from one such experiment. Five initially subthreshold second root stimuli were presented at 1/5 sec and the amplitude of the resulting e.p.s.p.

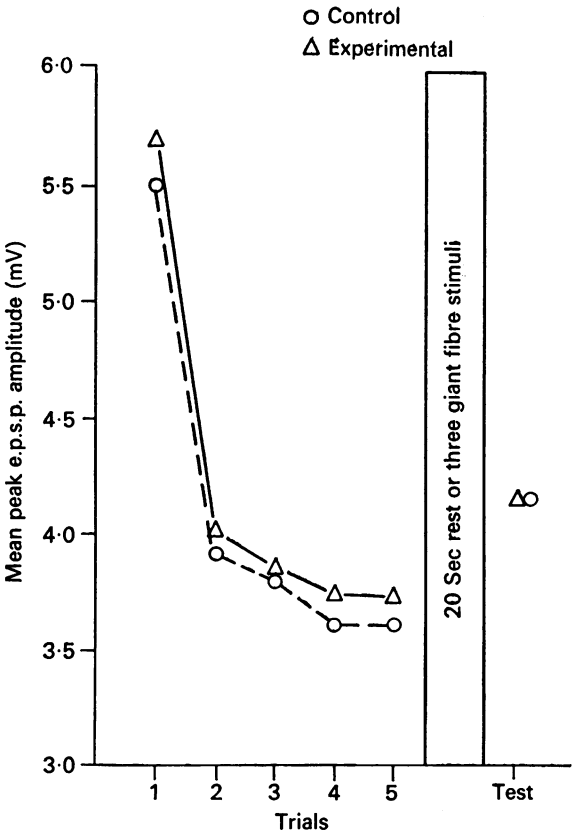

Fig. 5. Lack of effect of giant fibre spikes upon recovery from synaptic depression. Compound e.p.s.p.s to second root stimulation at 1/5 sec were recorded in the axon of the right lateral giant fibre in the 4th abdominal ganglion. In the control condition (open circles), 5 second root stimuli were followed by 20 sec rest and then by a single second root test shock. In the experimental condition (open triangles), 5 second root stimuli were followed by three suprathreshold stimuli to cord giant fibres at 1/5 sec and then 5 sec later by a single root test shock. Second root stimulus intensity was constant throughout. Control points are means of four runs; experimental points are of three runs.

in the lateral giant measured. Following the 5th second root shock there occurred either: three giant fibre stimuli at 1/5 sec (Fig. 5, experimental); or a 20 sec rest (Fig. 5, control) with no stimuli (thereby equating the time

required to administer the three giant fibre stimuli). Comparison of the mean amplitudes of the e.p.s.p.s on the test trials shows that there was no difference in recovery from the effects of the series of second root shocks whether giant fibre spikes or rest intervened between the last conditioning trial and the recovery test. Each point is the mean of several runs (control, $n = 4$; experimental, $n = 3$).

Protection of the first synapse from synaptic depression

The experiments reported above indicate that use-induced decline in efficacy of transmission through the afferent limb of the lateral giant reflex pathway can, under certain conditions, be largely avoided. Since such decline is thought to be due to homosynaptic depression at the first synapse

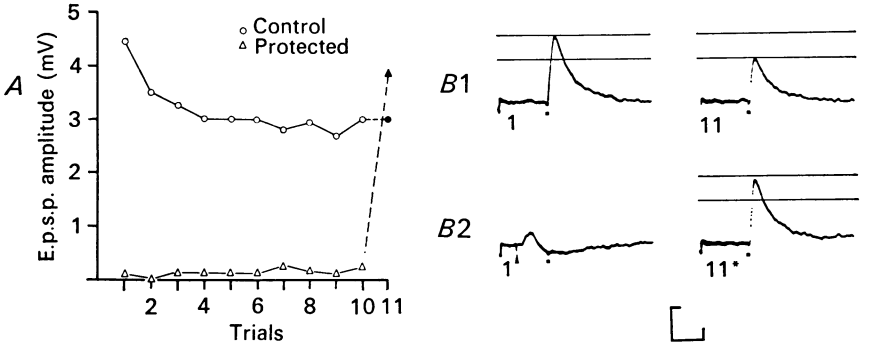

Fig. 6. Protection from synaptic depression at interneurone A. *A*, compound e.p.s.p. amplitude on a series of eleven first root stimuli at 1/4 sec. Control curve (open circles): responses to first root stimuli alone. Protection curve (open triangles): responses to first root stimulus presented 20 msec after suprathreshold stimulus to cord giant fibres (open triangles) and to a final first root stimulus given alone (filled triangle). Each point is a mean of three runs. First root stimulus intensity was constant throughout. *B*1, compound e.p.s.p.s on trial 1 and trial 11 of one of the control series plotted in *A*. *B*2, compound e.p.s.p.s on trial 1 and 11* of one of the protection series plotted in *A*. Giant fibre stimulus delivered at arrow; first root stimulus at square. Calibration: 2 mV and 20 msec.

of the reflex (Zucker, 1972*a*,*b*), we presumed that the protection seen in the lateral giant must be a reflexion of protection occurring at the first synapse. To verify this, intracellular recording was carried out from interneurone A (see Fig. 1), the largest first-order tactile interneurone in the lateral giant reflex pathway. This tactile interneurone receives monosynaptic chemical excitatory input in the sixth abdominal ganglion from mechanoreceptor hairs located on the ipsilateral telson and uropods (Kennedy, 1971; Zucker *et al.* 1971; Zucker, 1972*a*), and its rostrally

coursing axon forms excitatory electrical synapses with the lateral giant in the fifth and more rostral abdominal ganglia.

An electrical stimulus to any of roots 1 to 5 of the last abdominal ganglion activates tactile afferent fibres which synapse with interneurone A (Kennedy, 1971; and Fig. 6*B*1). Repetition of such stimuli results in a substantial decline over trials in the amplitude of the resulting compound e.p.s.p. In Fig. 6*A* (open circles), for example, ten stimuli to the first root at 1/4 sec resulted in an average decline in e.p.s.p. amplitude of approximately 32 %. The first and 11th trials of such a series are shown in Fig. 6*B*1. Mean percent e.p.s.p. declines over 11 trials at indicated interstimulus intervals are shown in Table 2 (column II) for five experiments at the first synapse. These data confirm results published previously (Krasne & Bryan, 1973; Zucker, 1972*a*,*b*; Zucker *et al.* 1971) showing that the first synapse of the lateral giant pathway is subject to depression.

TABLE 2. Declines of compound e.p.s.p.s recorded from interneurone A

		III							
	II	Experimental declines (as % of control declines)							
I	Control	Giant fibre-first root interstimulus interval (msec)							
Experiment	declines	0–15	16–30	31–45	46–60	61–75	76–90	91–105	106–120
J34	31·3 ($n = 4$)	—	30·7 ($n = 3$)	—	—	70·7 ($n = 2$)	64·1 ($n = 1$)	—	—
J40	29·6 ($n = 5$)	—	0·0 ($n = 2$)	—	0·0 ($n = 1$)	79·1 ($n = 1$)	—	24·7 ($n = 1$)	—
J41	37·2 ($n = 12$)	—	40·1 ($n = 2$)	—	47·3 ($n = 2$)	—	89·1 ($n = 3$)	—	91·5* ($n = 2$)
J42	25·7 ($n = 6$)	—	13·1 ($n = 3$)	—	—	—	—	59·0 ($n = 2$)	—
J43	47·8 ($n = 5$)	—	13·2 ($n = 3$)	—	40·0 ($n = 1$)	30·3 ($n = 2$)	—	107·3 ($n = 1$)	96·5 ($n = 1$)

— Indicates interval not tested. * One point was omitted from the mean (% experimental decline = 143 % of control decline). See text for reasons for omission. Interstimulus intervals: J34, 1/4 sec.; J40, J41, J42 and J43, 1/3·2 sec.

The experimental design for testing for protection was identical to that used in experiments at the level of the lateral giant. Fig. 6 illustrates an experiment in which afferent root shocks (the first root of last ganglion) were given at 4 sec intervals and tail-flip motor circuitry was activated by direct stimulation of either lateral or medial giant 20 msec before the afferent stimuli on experimental conditioning trials. Each point in A is a mean of three runs. Fig. 6*B* shows typical e.p.s.p.s from the same experiment. Comparison of e.p.s.p. amplitudes on trials 11 and 11* of control and

experimental series, respectively, shows that preceding afferent root shocks by giant fibre stimulation resulted in substantial protection.

As was the case at the level of the lateral giant, giant fibre firing produces inhibitory events which reduce afferent root evoked e.p.s.p.s on experimental series conditioning trials; this inhibition is associated with a diphasic post-synaptic potential in interneurone A (Fig. 6 $B2_1$), the hyperpolarizing portion of which is an i.p.s.p. (further discussion of this inhibition is deferred to the following paper). Because of these effects, quantitative

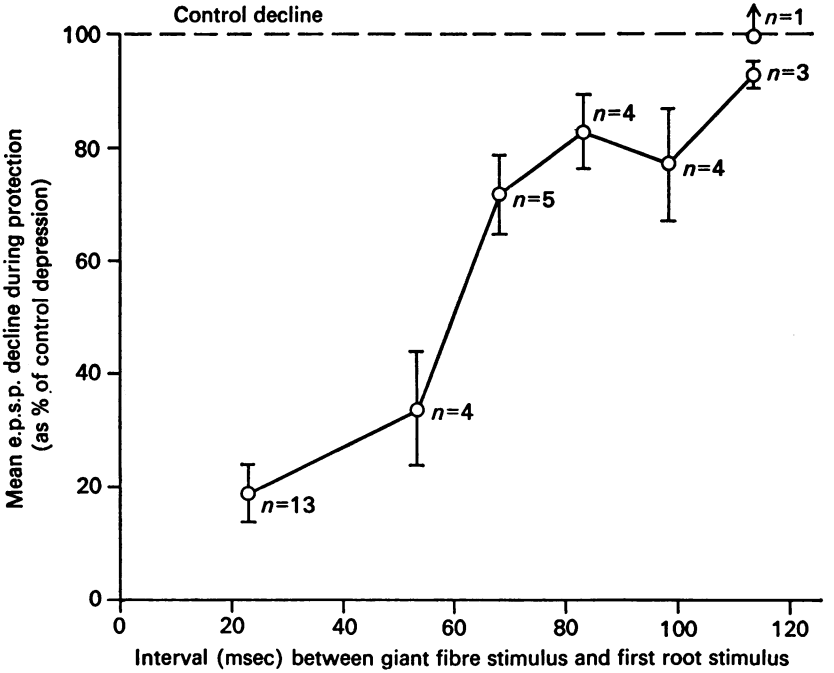

Fig. 7. Mean % e.p.s.p. decline at interneurone A during protection, expressed as the percent of the mean decline occurring on adjacent control runs. Protection series consisted of ten stimuli to the first root of the last abdominal ganglion at 1/3·2–4 sec with each first root stimulus following a single suprathreshold giant fibre shock at a specified interval. On control runs, first root stimuli were presented alone at the same frequency and intensity as on protection runs for that preparation. Data for all runs at a given first root/giant fibre interval were pooled between preparations. Not all intervals were tested in each experiment (see Table 2). Each error marker is the S.E. of the mean of the pooled data at that interval.

measures of protection were computed by the same rules used in the lateral giant experiments. In the experiment of Fig. 6 the control series e.p.s.p. decline was 32 %, while the experimental series decline was only

11·4 %. The experimental series decline was therefore 35·8 % of the control series decline, and the percentage protection was 64·2 %. Data from five experiments in which inter-trial intervals were from 3·2 to 4·0 sec and a range of giant fibre-to-afferent root intervals were examined are given in Table 2. Mean percent protection ranged in different preparations from 59 to 100 % when giant fibre firing preceded afferent root shocks by 16–30 msec.

The time course of protection at the first synapse is plotted in Fig. 7 (based upon experiments in Table 2). Giant fibre–sensory root interstimulus intervals of from 16 to 120 msec were examined (giant fibre stimuli always preceded root stimuli in these experiments). For convenience in pooling the results obtained in five separate experiments, the range of tested interstimulus intervals was divided into 15 msec time bins, and a mean and standard error of the mean for all runs within that bin were calculated. E.p.s.p. decline over a series of ten conditioning stimuli is minimum when the afferent root stimuli occur in the interval from 16 to 30 msec after impulse activity in cord giant fibres, being reduced to about 20 % of that occurring when afferent stimuli are presented alone. Protection remained strong 60 msec after giant fibre activity, and was reduced but still present from 61 to 105 msec after a giant fibre spike (the mean decrement for the thirteen runs in this interval being 77 % (S.E. of mean = 4·9) of the decrement on appropriate control runs). Three out of four runs* in the longest interval examined suggested the persistence of a weak protection effect as late as 106–120 msec, although more runs at these long interstimulus intervals would be necessary to establish this point with certainty.

It should be emphasized that the protection effect at the first synapse was highly consistent, occurring in all but one out of thirty determinations with interstimulus intervals of 16–105 msec.

DISCUSSION

We have shown here that the decline of lateral giant escape reflex excitability normally produced by repetitive afferent stimulation is greatly diminished if the afferents are stimulated shortly after activation of tail-flip motor circuitry, a phenomenon we have termed 'protection'. Moreover, we have shown that this protection applies to homosynaptic depression between the tactile afferents and the largest of the sensory interneurones intercalated between the afferents and the lateral giant command cell.

We also noted that protection is associated with inhibition at both the

* In Fig. 7 one run was plotted separately in the 106–120 msec interval because the occurrence of a considerably *augmented* decline (143 % of control) on an experimental run was contrary to all other data we obtained.

first and second synapses of the lateral giant reflex. Taken together with the fact that synaptic depression is often thought to be due to transmitter depletion or post-synaptic receptor desensitization, this suggests the hypothesis that protection is due to presynaptic inhibition of the sensory afferents for the reflex. This hypothesis will be considered in the following paper.

We believe that protection allows an animal to tail-flip without habituating its lateral giant escape reflex despite stimulation of tactile receptors caused by the animal's own tail-flip movements. The time course of protection (which starts about 10 msec after giant fibre firing, is strong for some 30–40 msec, and is over at about 100 msec) is commensurate with the time required for a tail-flip; flexion begins at about 10 msec, is complete in about 30 msec, and is followed by re-extension which requires some 50 msec (Roberts, 1968; Wine & Krasne, 1972). We have not attempted to verify that tail-flips actually cause firing of tactile afferents, because it would be difficult to record from the afferents during a tail-flip; in view of the extreme sensitivity of the afferents, it seems virtually certain that a tail-flip would evoke massive firing. Our interpretation of the present observations is strengthened by the fact that behavioural habituation of tail-flips that are evoked by tactile stimuli is greatly diminished if the stimuli are presented during tail-flips evoked by direct giant fibre stimulation (Wine, Krasne & Chen, 1975). We also have not verified that non-giant-mediated tail-flips (Schrameck, 1970) result in protection, but we do know that interneurones that produce presynaptic inhibition of tactile afferents fire during non-giant tail-flips (Krasne, Wine & Kramer, 1977).

Protection from habituation to stimuli that are produced by an animal's own movements is probably common. Wiersma & Yamaguchi (1967) found that the 'jittery movement' detectors of the crayfish visual system, which respond to moving objects in the visual field but habituate rapidly, do not habituate when visual field movements are produced by an animal's active movements of its own eye stalks. More recently, O'Shea & Rowell (1975) have reported a comparable protection phenomenon in descending movement detector neurones of the locust; in this case protection occurs whenever the entire visual field moves, even when such movement is not produced by locomotion. Also perhaps comparable is the observation that in humans the transient auditory threshold increases that follow periods of loud sound are less if, during the sound, the person continuously vocalizes; this effect is believed to depend partly on central factors (Benguerel & McBay, 1972).

We emphasize that the *prevention* of the processes responsible for habituation differentiates protection from *sensitization* (Carew, Castellucci & Kandel, 1971; Thompson & Spencer, 1966) or *dishabituation* (Rowell,

1971). Sensitization does not prevent the occurrence of habituation but only compensates for it by overlaying facilitatory processes which, when they dissipate, leave the preparation still habituated. True dishabituation, in contrast to both sensitization and protection, resets the system to a state that seems to be identical to that existing prior to habituation. All these phenomena may function to mitigate against maladaptive consequences of habituation, but each presumably has somewhat different circumstances under which its occurrence is most appropriate.

The present case of protection from habituation is specifically due to prevention of the synaptic depression which underlies the habituation. It is also known that development of temporal facilitation of neuromuscular transmission in crayfish claw opener muscles can be diminished by preceding each motor neurone spike by firing of the muscle's peripheral inhibitor (Dudel & Kuffler, 1961*b*). And it has recently been shown that the courses of synaptic depression, post-tetanic potentiation, and temporal facilitation at synapses on R15 of *Aplysia* abdominal ganglion can be altered by bath application of dopamine or by stimulation of the branchial nerve (Tremblay *et al.* 1976; Woodson, 1976). Taken together, such observations suggest that the development of use-induced changes of synaptic efficacy might often be subject to modulation.

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