J. exp. Biol. (1981), 91, 145–164
With 9 figures
Printed in Great Britain

ACTIVITY OF EXCITOR AND INHIBITOR CLAW MOTOR NEURONES DURING HABITUATION AND DISHABITUATION OF THE CRAYFISH DEFENCE RESPONSE

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(*Received* 2 *May* 1980)

SUMMARY

1. The activity of the opener excitor and inhibitor motor neurones was recorded during habituation and dishabituation of the defensive claw opening response in partially restrained crayfish (*Astacus* sp.).

2. Claw opening evoked by brief tactile stimulation of the thorax undergoes habituation if the stimulation is repeated at 30 s or 1 min intervals. This habituation is accompanied by a progressive decrease in evoked excitor activity, while the activity of the inhibitor remains unchanged.

3. The habituated claw opening response can be dishabituated by tactile stimulation of the claw or head, or by visual stimulation. Dishabituation is accompanied by both an increase in evoked excitor activity and a decrease in evoked inhibitor activity.

4. Dishabituation is also accompanied by potentiation of claw opening: that is, the same number of excitor and inhibitor spikes produce greater claw opening following dishabituating stimulation.

5. The potentiation of claw opening following dishabituation is due in part to post-tetanic potentiation (PTP) at the excitor neuromuscular junction. PTP was demonstrated with physiological parameters of stimulation in isolated claw preparations, and is of sufficient magnitude and duration to account for the observed potentiation.

6. These results contradict the conclusion from an earlier study (see Schöne, 1961) that habituation of the claw opening response is due to an increase in inhibitor activity. They also provide new evidence for a role in dishabituation of both disinhibition and PTP.

INTRODUCTION

In principle, both habituation and dishabituation may be due to changes in either neural excitation or inhibition. Pavlov (1927) attributed habituation (which he referred to as extinction) to increased inhibition, and dishabituation to disinhibition.

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Many later theorists (e.g. Sokolov, 1963; Konorski, 1967) have maintained t[redacted] position on habituation, which has received additional support from some physiological studies (Wickelgren, 1967*a*, *b*; Wall, 1970; MacDonald & Pearson, 1979*a*, *b*). Studies on preparations amenable to detailed cellular analysis, however, have indicated that habituation is due to an intrinsic decrease in neural excitation (Spencer, Thompson & Nielson, 1966; Castellucci *et al.* 1970; Zucker, 1972), and dishabituation to Castellucci *et al.* 1970).

The crayfish defensive claw opening response has several advantages for investigating the role of excitation and inhibition in habituation and dishabituation. Claw opening is produced by one muscle (the dactyl abductor) which is innervated by only two motor axons, one excitor and one inhibitor, which run in different nerve bundles (Van Harreveld & Wiersma, 1937). The final site of integration is thus particularly accessible. During the course of studies which first established the existence of peripheral inhibition in Crustacea, Hoffman (1914) found that cutting the nerve bundle which contains the axon of the opener inhibitor for one claw abolished habituation of opening of that claw in response to taps on the back or tail, while opening of the other (unoperated) claw habituated normally. Cutting the opener inhibitor for the second claw subsequently abolished habituation of its response as well (the closer tendons were cut in both claws).

Hoffmann's results have been cited (Schöne, 1961) as demonstrating that habituation of the claw opening response is due to a build-up of peripheral inhibition. Since such a mechanism of habituation has not yet been clearly demonstrated in other preparations, we have examined this hypothesis using electrophysiological methods. We have also investigated whether dishabituation is due solely to an increase in excitation or whether other mechanisms contribute to it as well.

METHODS

Experiments were performed on mature crayfish (*Astacus* sp.) of both sexes, obtained from a local supplier. The animals were housed in an aquarium containing circulating, oxygenated water, and were fed twice a week. Experimental subjects were selected for a vigorous defence response and used within 2 weeks of arrival. Two types of preparations were used: the whole animal and the isolated claw.

Whole animal preparation. Animals were strapped to a piece of plexiglass in the form of a Y and bathed in shallow tap water cooled to around 15 °C during the experiments (Fig. 1). The animals were anaesthetized for surgery by brief immersion in ice water. A thin nerve containing only the axons of the two dactyl opener motor neurones was exposed just proximal to the opener muscle by cutting a small hole in the dorsal proximal end of the propodite. This procedure generally produced little or no muscle damage or bleeding. The activity of the opener motor neurones was recorded by picking the nerve up on two fine platinum hook electrodes into a film of paraffin oil. Muscle potentials were recorded differentially between two fine, insulated stainless steel wires inserted through the carapace over the dactyl opener and closer muscles (the dactyl closer muscle was almost always silent in these experiments).

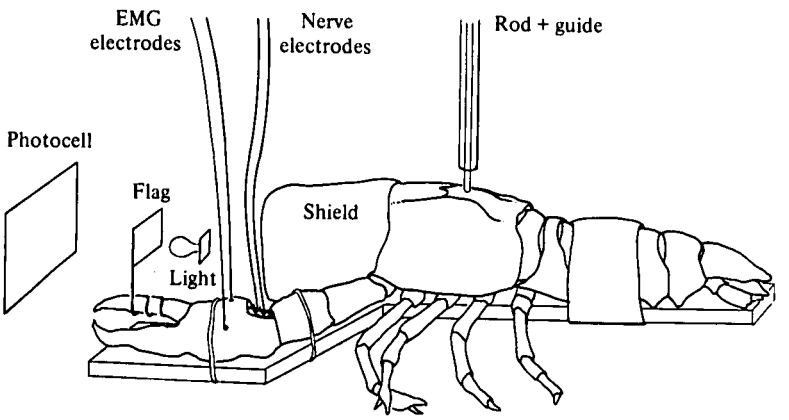

Fig. 1. Whole animal preparation (see the text for details).

Claw opening was evoked by dropping a 4 g rod from a measured height on to the dorsal thorax of the animal. The rod was guided in a fixed tube so that it always made contact at the same spot, and the time of contact was monitored electrically. The animal's eyes were shielded so that it could not see the rod or other tactile stimuli. Stimulation was delivered in series of trials and was the same for all trials in a series. There was generally a 30 s interval between trials and a much longer rest between series. Movement of the dactyl was recorded by attaching to it a small aluminium foil 'flag' which interrupted a light beam focused upon a silicon voltaic cell. The output of the voltaic cell was approximately proportional to the height of the dactyl opening. Sometimes small lead weights were attached to the tip of the dactyl to increase the load on the opener muscle. Signals were displayed on an oscilloscope and photographed.

Isolated claw preparation. The nerves were exposed in the meropodite of an autotomized cheliped by removal of the overlying exoskeleton and muscle. The dactyl closer tendon was cut and the dorsal surface of the dactyl opener muscle was exposed by cutting away the overlying cuticle. The preparation was placed in a small chamber containing crayfish saline (NaCl, 195 mM; KCl, 5·4 mM; $CaCl_2$, 13 mM; $MgCl_2$, 2·6 mM; buffered to pH 7·0–7·2 with 10 mM-Tris-Maleate) and cooled to around 12 °C. The opener excitor and inhibitor neurones were stimulated electrically with 0·1 ms negative-going pulses delivered through suction electrodes in the meropodite, where the axons of the two neurones run in different nerve bundles. The membrane potential of single opener muscle fibres was recorded intracellularly with 'floating' glass microelectrodes filled with 3 M-KCl. Recordings were made as for the whole animal preparation.

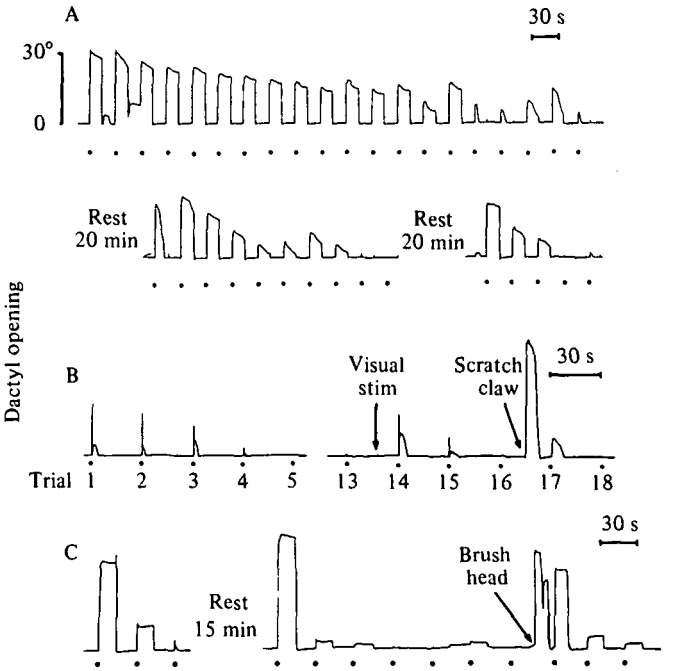

Fig. 2. Habituation and dishabituation of the claw opening response. (A) Habituation, recovery with rest, and more rapid rehabituation. A glass rod was dropped from a constant height on to the animal's dorsal thorax every 30 s (the times of stimulation are indicated by the dots). On most trials the claw was forcibly closed 15 s after the stimulus. (B) Dishabituation by repeated approach of an object in the animal's visual field (between trials 13 and 14) and by scratching the dorsal surface of the claw (between trials 16 and 17). In this series extra weights were attached to the dactyl so that it closed spontaneously. (C) Dishabituation by brushing the head.

RESULTS

Habituation and dishabituation of the claw opening response

Mechanical stimulation of the thorax (a single tap) elicited opening of the claws in the restrained, whole animal preparation. When this stimulation was repeated at 30 s or 1 min intervals, the amplitude of the evoked claw opening typically decreased (habituated) in a graded fashion. The opening response recovered with rest and rehabituated more rapidly during a subsequent series of trials (Figs. 2A, C). In a few experiments, habituation was more rapid with weak than with strong stimuli, and there was partial generalization of habituation between different loci of stimulation on the dorsal thorax and head.

The habituated claw opening response could be restored immediately (dishabituated) by stimulation of other parts of the body. Dishabituating stimuli included scratching the dorsal surface of the dactyl or propodite and tapping or brushing the head (both of which evoke reflex opening of the claws) as well as repeatedly moving an object towards the animal's head (Figs. 2B, C). This last stimulus may have a mechanical component (from displacement of air by the moving object) in addition to a visual component, but it is of interest because it does not itself elicit claw opening

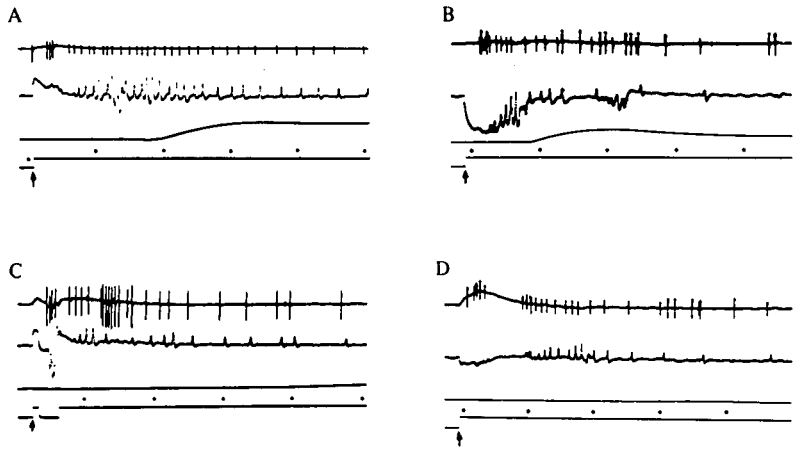

Fig. 3. Patterns of evoked motor neurone activity. (A) Basic pattern. Top trace: extracellularly recorded spikes of excitor (lower amplitude) and inhibitor (higher amplitude) motor neurones of the claw opener muscle. The spikes are retouched in this and subsequent figures. Second trace: electromyogram recorded differentially between the opener and closer muscles. Recording was AC for traces one and two, in which slow fluctuation are movement artifacts. Third trace: movement of the dactyl. Bottom trace: stimulus marker (upward step) and time calibration (100 ms between pulses). The arrow indicates the time of impact of the glass rod. The height of the time pulses gives the amplitude calibration for the nerve and muscle recordings, which is 100 μV for most records. (B) Second inhibitor response after the onset of movement and phasic opening. (C) Second inhibitor response before the onset of movement. (D) Pause in evoked excitor activity.

in our experimental conditions. With all of these stimuli, dishabituation was typically brief, lasting at most a few trials.

These observations indicate that habituation of the claw opening response is similar in many respects to habituation of other responses in a wide variety of species (Thompson & Spencer, 1966). In addition, claw opening sometimes became considerably larger in amplitude on the second trial of a series before subsequently habituating (this phenomenon has been described for other reflexes, cf. Groves & Thompson, 1970). In several instances, claw opening became longer in duration during a series of trials (see Hawkins & Bruner, 1979).

Pattern of evoked motor neurone activity

It was possible to record the activities of the opener motor neurones in the partially restrained animal without noticeably altering the behaviour of the animal. The average spontaneous firing rates of the excitor and inhibitor neurones were 0–1 and 0–3 spikes/s, respectively. Fig. 3A shows the basic pattern of the evoked activity in these neurones during the first 500 ms following brief mechanical stimulation of the thorax. The average latency of firing of both the excitor and inhibitor neurones was about 20 ms. In rested preparations, the excitor usually began firing at about 100 Hz and its rate of firing decelerated gradually over a few seconds. The inhibitor fired an initial burst of two or three spikes at 200 Hz and then went silent for around 100 ms. During this period, there were never any inhibitor spikes, even

in those preparations in which the spontaneous rate of inhibitor firing before stimulation was sufficiently high that some would have been expected. After this silent period the inhibitor fired either above or below its spontaneous rate for the remainder of the opening. The latency of the first dactyl movement was 100–200 ms, and the latency to maximum dactyl opening was generally less than 500 ms (Fig. 3A).

The pattern shown in Fig. 3A occurred only when the dactyl was relatively heavily weighted (1 g or more). When the dactyl was weighted less heavily or not at all there was a delayed inhibitor response of lower frequency in addition to the short latency inhibitor burst. This second inhibitor response usually occurred after the dactyl movement had begun (Fig. 3B), and thus may depend on proprioceptive feedback.

Fig. 3B also illustrates a very phasic claw opening during which downward deflections which probably represent closer muscle potentials are evident in the EMG record at the peak of the movement. Closer muscle potentials were not observed during most claw openings, which typically were of much longer duration than the one shown in this figure.

Fig. 3C shows a less common variation in which a second inhibitor response occurred *before* opening had started. This pattern was observed only on trials in which there was an early downward deflection in the EMG record which may represent fast closer activity. Since this pattern also occurred in one preparation in which both motor neurones had accidentally been killed at the recording electrodes, the entire pattern is probably pre-programmed and not due to proprioceptive feedback. Finally, Fig. 3D shows another less common variation in which there was an early excitor burst followed by a brief pause, much like the inhibitor pattern. In general, the firing pattern was usually the same on all trials in a series and varied both with amount of dactyl weight and between animals.

Of the series of trials described below, pattern A occurred in three, B in fourteen, C in one, and D in seven.

Changes in motor neurone activity accompanying habituation

We recorded the activities of the excitor and inhibitor motor neurones during 25 series of trials in six whole animal preparations (series are included in the results only if they consisted of at least five trials and claw opening was at least 4° on the first trial). In order to get an index of habituation during these series, we compared the median angle of claw opening during the first and second halves of each series, excluding any trials after attempted dishabituation. Claw opening was significantly smaller during the second halves of the series than during the first (t for paired observations = 5·10, $P < 0{\cdot}01$). Claw opening decreased during 23 of the 25 series, and in each of the six animals. Thus, habituation of claw opening was a highly reliable phenomenon in this preparation.

Figs. 4 and 5 show examples of the evoked motor neurone activity during habituation of claw opening in response to mechanical stimulation of the thorax, recovery with rest, and during dishabituation by brushing the head or scratching the dactyl. Since claw opening was generally complete within 500 ms following the stimulus, we have used the total number of spikes in that period as a measure of evoked motor neurone

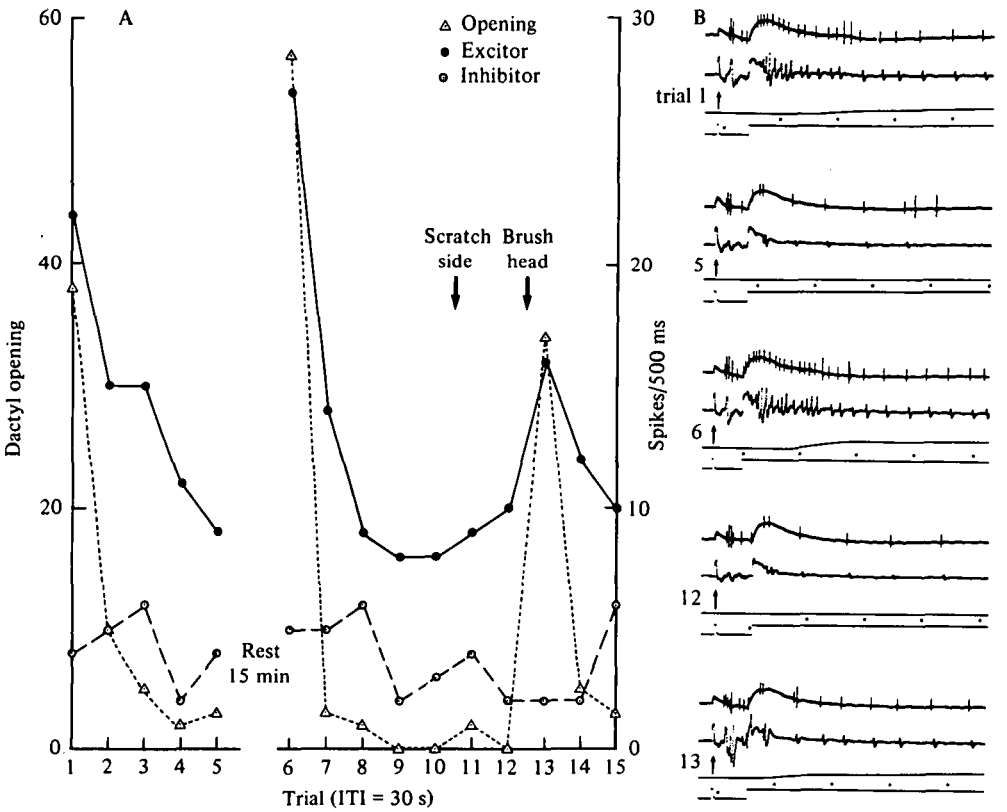

Fig. 4. Evoked motor neurone activity during habituation and dishabituation by brushing the head. (A) Graphs of the maximum claw opening in degrees (triangles) and number of excitor (filled circles) and inhibitor (open circles) spikes evoked during the first 500 ms by brief mechanical stimulation of the thorax at 30 s intervals. Between trials 10 and 11 the animal's lateral thorax was scratched and between trials 12 and 13 its head was brushed. (B) Records as in Fig. 3 of the evoked motor neurone activity during the first 500 ms of selected trials from the same series as shown in part A.

activity. In each of these series of trials, habituation of the claw opening response was accompanied by a decrease in the number of excitor spikes, whereas the number of inhibitor spikes remained roughly constant. There was no evident change in the pattern of evoked motor neurone activity accompanying habituation, other than a decrease in the average frequency of excitor firing.

Table 1 summarizes the changes in motor neurone activity which occurred in all 25 series of trials in six animals. In order to get an index of changes in motor neurone activity accompanying habituation, we compared the median number of evoked excitor and inhibitor spikes during the first and second halves of each series. The number of evoked excitor spikes was significantly less during the second halves of these series than during the first ($t = 5{\cdot}94$; $P < 0{\cdot}01$). Evoked excitor activity decreased in 24 of the 25 series, and in each of the six animals. By contrast, there was no consistent change in the evoked inhibitor activity, nor was there any change in the background firing rates of either the excitor or the inhibitor measured during

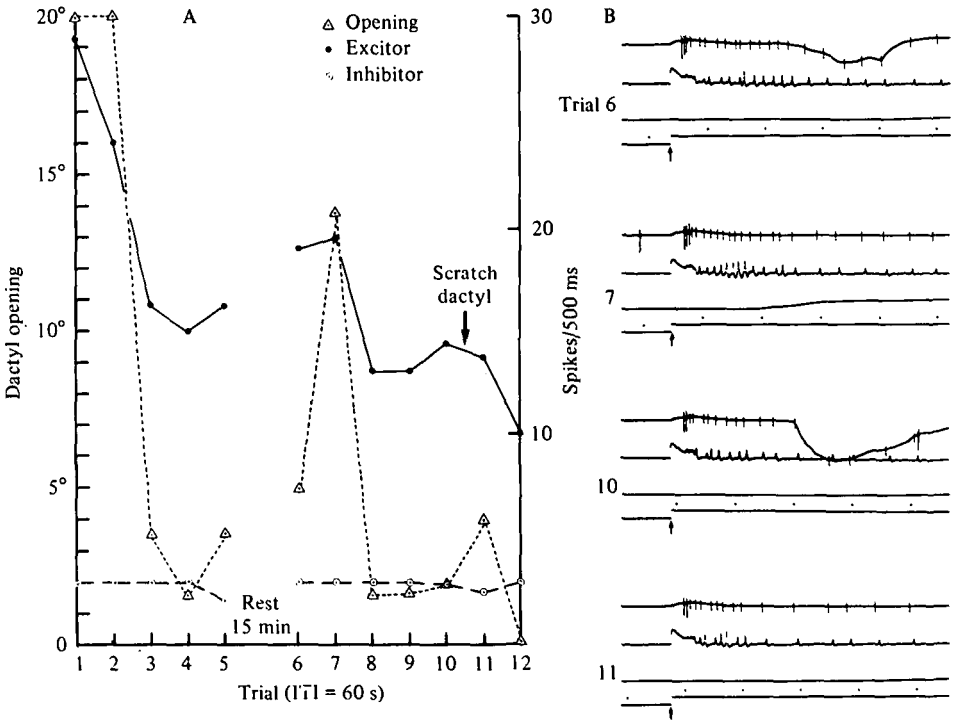

Fig. 5. Evoked motor neurone activity during habituation and dishabituation by scratching the dactyl (same format as Fig. 4).

the 2 s before each stimulus. Similar results were obtained with each of the four patterns of motor neurone activity. Thus, at the level of the motor neurones, habituation of the claw opening response is due to a decrease in evoked excitor activity and not to an increase in either evoked or background inhibitor activity. This conclusion is further supported by the observation that habituation occurred normally in one animal in which the inhibitor axon was damaged during the dissection and did not conduct spikes.

Changes in motor neurone activity accompanying dishabituation

We recorded the activities of the excitor and inhibitor motor neurones during nine successful dishabituations of the claw opening response (dishabituation was said to have occurred if the angle of opening on the trial following the dishabituation attempt was at least one-third larger than it had been on any of the three preceding trials). At least one successful dishabituation was obtained with each of the six animals. The stimuli which produced dishabituation in these preparations were scratching the dactyl or propodite in five cases, and tapping or brushing the head in four cases.

Figs. 4 and 5 show examples of the evoked motor neurone activity on the trials before and after dishabituation of the claw opening response. In the example shown in Fig. 4 there was an increase in the number of excitor spikes and no change in the number of inhibitor spikes accompanying dishabituation by brushing the head (this figure also shows a failure to dishabituate by scratching the thorax). In Fig. 5

Table 1. *Changes in number of excitor (E) and inhibitor (I) spikes accompanying habituation*

	Number of spikes during the 2 s before the stimulus		Number of spikes during the 500 ms after the stimulus		Claw opening (degrees)
	E	*I**	*E*	*I**	
Average during the 1st half of each series of trials	0·62	3·31	16·12	5·97	12·02
Average during the 2nd half of each series of trials	0·90	2·94	11·76	5·72	4·46
Average difference	0·28	−0·37	−4·36	−0·25	−7·56
S.E. difference	0·29	0·41	0·73	0·34	1·48
P	n.s.	n.s.	< 0·01	n.s.	< 0·01
No. +	4	4	1	4	0
−	2	7	24	8	23
0	19	7	0	6	2

* In one animal the inhibitor axon was damaged during the dissection and did not conduct any spikes.

Table 2. *Changes in number of excitor (E) and inhibitor (I) spikes accompanying dishabituation*

	Number of spikes during the 2 s before the stimulus		Number of spikes during the 500 ms after the stimulus		Claw opening (degrees)
	E	*I**	*E*	*I**	
Average on the trial pre-dishabituation	0·56	2·50	10·22	6·25	2·34
Average on the trial post-dishabituation	1·56	4·00	12·67	4·50	14·20
Average difference	1·00	1·50	2·45	−1·75	11·86
S.E. difference	0·53	1·45	0·99	0·67	3·31
P	n.s.	n.s.	< 0·05	< 0·05	< 0·01
No. +	5	3	6	0	9
−	1	2	2	6	0
0	3	3	1	2	0

* In one animal the inhibitor axon was damaged during the dissection and did not conduct any spikes.

a slight decrease may be seen in the number of both excitor and inhibitor spikes accompanying dishabituation by scratching the dactyl. In neither example was there any obvious change in the pattern of evoked motor neurone activity accompanying dishabituation, other than changes in the average spike frequencies.

Table 2 summarizes the changes in motor neurone activity which accompanied the nine successful cases of dishabituation in six animals. There were both significantly more evoked excitor spikes ($t = 2{\cdot}47$, $P < 0{\cdot}05$) and significantly fewer evoked inhibitor spikes ($t = 2{\cdot}61$, $P < 0{\cdot}05$) on the trials following dishabituation, compared to the trials preceding dishabituation. There was no significant change in the spontaneous firing rate of either the excitor or inhibitor, measured during the two seconds preceding mechanical stimulation of the thorax. Thus, dishabituation of the claw

opening response is due at least in part to changes in the evoked activities of both the excitor and inhibitor motor neurones.

Relation between degree of claw opening and motor neurone activity

In some cases the changes in motor neurone activity accompanying dishabituation seem inadequate to account for the observed change in claw opening. In the example shown in Fig. 5, for instance, there was a slight *decrease* in the number of excitor spikes following the dishabituating stimulus and yet the degree of claw opening doubled. Similarly, there was a large increase in claw opening on the second trial of this series with very little increase in evoked excitor activity. In order to assess such apparent discrepancies between changes in claw opening and changes in motor neurone activity, however, it is first necessary to know the normal relation between degree of claw opening and number of excitor and inhibitor spikes.

The equation we have used is $\theta = K_1 (E-K_2)/(I-K_3)$ where θ is claw opening in degrees, E and I are the number of excitor and inhibitor spikes in the first 500 ms, and K_1, K_2 and K_3 are positive constants. This relation was suggested to us by previously published data obtained with a similar experimental arrangement (Smith, 1975). The BMDPAR curve-fitting program (Dixon & Brown, 1977) was used to estimate K_1, K_2 and K_3 for each subject and each amount of dactyl weight (a total of nine data sets), based on the data from all trials with measurable claw opening. This curve-fitting procedure accounted for over 80% of the variance in five of the nine data sets, for 50–80% in two, and for less than 50% in the two data sets in which closer muscle activity was observed. These results show that the equation we used was generally quite successful in describing our data, and they also support the idea that there was no covert closer muscle activity in the seven data sets in which it was not observed. The average values of K_1, K_2 and K_3 for those data sets with no dactyl weight were 15·7, 6·6 and 8·0 respectively. These values are in at least rough agreement with those we estimated from the data of Smith (1975), which were 9, 5 and 11. Adding dactyl weight increased K_2 in all cases (three), while it had less consistent effects on the other parameters.

Potentiation of claw opening following dishabituation

Fig. 6 shows a graph comparing the observed claw opening on each trial with the opening predicted by the equation $\theta = K_1(E-K_2)/(I+K_3)$ using the values of the parameters K_1, K_2 and K_3 estimated by the curve-fitting routine. The points on this graph tend to cluster around the diagonal line, indicating a good correlation between degree of claw opening and motor neurone activity. Eight of the nine data points from the trials following successful dishabituation of the claw opening response (arrowed) are above the diagonal – that is, the observed opening in those trials was greater than would have been predicted from the number of excitor and inhibitor spikes, compared to other trials from the same subject. We shall refer to this difference between observed and predicted claw opening as potentiation. The average potentiation of opening in the trials following successful dishabituation was 56%. Claw opening tended to be greater than the predicted value not only in the trials following successful dishabituation ($t = 2{\cdot}67$, $P < 0{\cdot}05$), but also in the trials following *all* dishabituation attempts, successful or not ($t = 1{\cdot}96$, $p < 0{\cdot}05$ one tail).

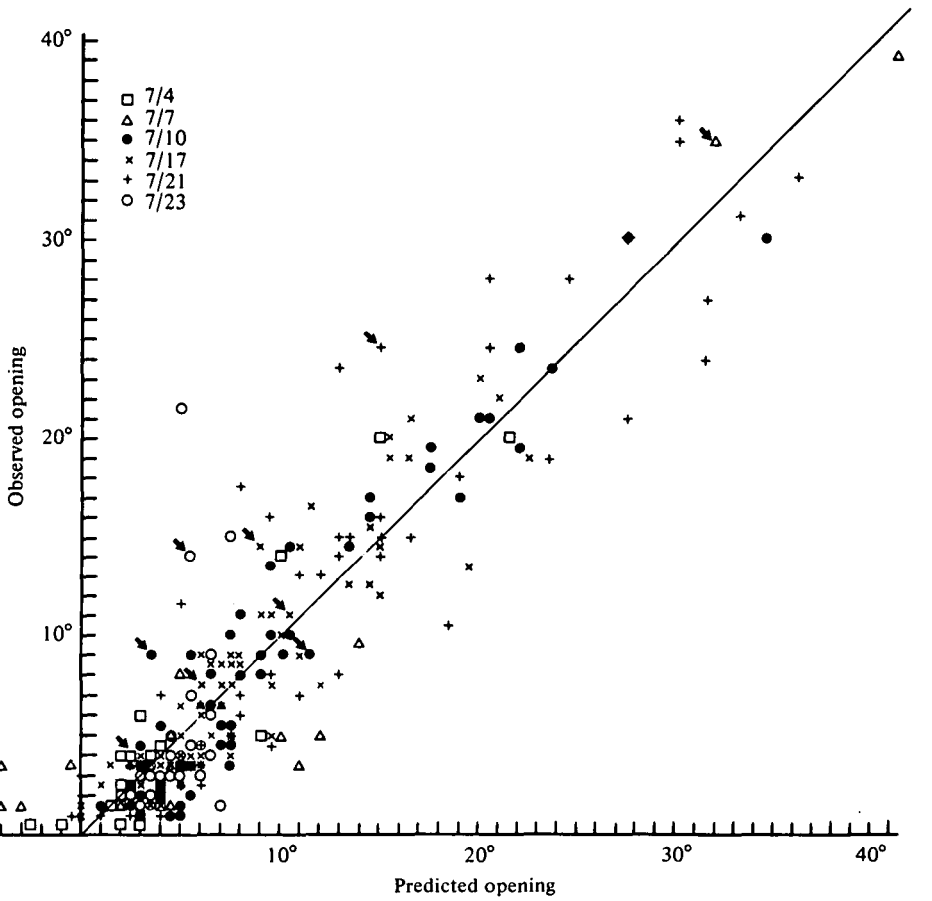

Fig. 6. Potentiation of claw opening following dishabituation. The points show the observed opening and the opening predicted from the number of excitor and inhibitor spikes on each trial, for each subject. The different symbols indicate the data points from the six different subjects (the numbers refer to the date on which each subject was run). The arrows identify the nine points from the trials immediately following successful dishabituations of the claw opening response.

Another way of looking at these data is to compare the observed and predicted *change* in claw opening from the trial before to the trial after dishabituation. In every one of the nine successful cases of dishabituation the increase in claw opening was greater than would have been predicted from the change in motor neurone activity. In fact, in two cases, claw opening increased despite a decrease in the predicted opening (Fig. 5).

Claw opening was also greater than the predicted value in the second trial of all five series in which opening in that trial was greater than in the first. However, there was no significant difference between observed and predicted opening when all second trials were considered. Thus, although potentiation of claw opening on the second trial was quite dramatic in a few cases (as in Fig. 5), it was not a reliable phenomenon and we have not attempted to analyse it further.

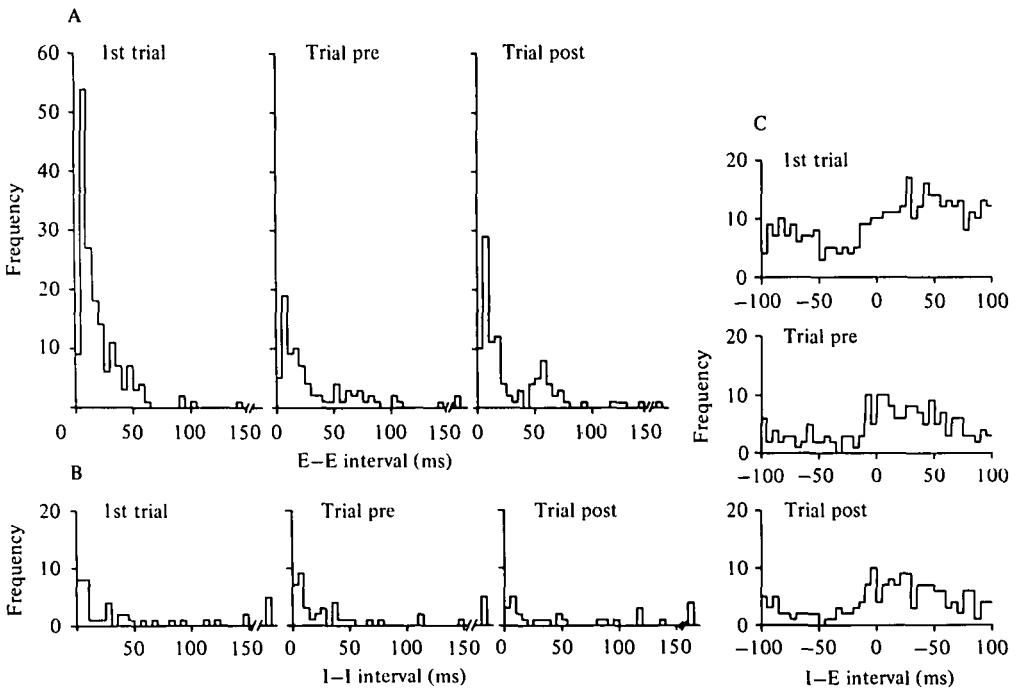

Fig. 7. Interspike intervals before and after habituation and dishabituation. (A) Frequency histograms of the intervals between successive excitor spikes during the first 500 ms of the first trial, the trial before dishabituation and the trial after dishabituation in the nine series of trials with successful dishabituation of the claw opening response. (B) Frequency histograms of the intervals between successive inhibitor spikes during the same periods. (C) Frequency histograms of all inhibitor-excitor intervals within the range ±100 ms during the same periods.

Changes in interspike intervals accompanying habituation and dishabituation

Fig. 6 indicates that the changes in number of excitor and inhibitor spikes accompanying dishabituation are not sufficient to account quantitatively for the observed increase in claw opening. One possible explanation of this discrepancy would be a change in the pattern of excitor and inhibitor activity accompanying dishabituation. We have not observed any gross change in pattern following dishabituation, but more subtle changes could be important. For instance, pairs of excitor or inhibitor spikes are more effective than equally spaced spikes with the same average frequency (Ripley & Wiersma, 1953). Likewise, the effectiveness of inhibitor spikes is critically dependent on the inhibitor-excitor interval, being maximal at 0–10 ms (Marmont & Wiersma, 1938; Dudel & Kuffler, 1961*b*). Thus relatively small changes in the timing of excitor or inhibitor spikes might contribute to dishabituation and possibly also to habituation of the response.

We have tested these possibilities by measuring the interspike intervals during the first 500 ms of the first trial, the trial before dishabituation, and the trial after dishabituation in the nine series of trials with successful dishabituation of the claw opening response. Fig. 7A shows frequency histograms of the intervals between successive excitor spikes, Fig. 7B of the intervals between successive inhibitor

kes, and Fig. 7C of all inhibitor-excitor intervals within the range ± 100 ms in those trials. Changes in the distribution of excitor-excitor, inhibitor-inhibitor, and inhibitor-excitor intervals accompanying habituation and dishabituation were analysed statistically by chi-squared tests using a 25 ms bin width.

The distribution of excitor-excitor intervals changed significantly accompanying habituation of the response: in the first trial there were relatively more intervals that lasted less than 50 ms and relatively fewer that lasted longer, compared to the trial before dishabituation ($\chi^2 = 20{\cdot}8$, $P < 0{\cdot}01$). This change in the distribution of excitor-excitor intervals is a consequence of a change in the initial frequency of excitor firing, which became progressively less in later trials of a series.

In contrast to habituation, there was no significant change in the relative distribution of excitor-excitor intervals accompanying dishabituation. There was also no significant change in the relative distribution of inhibitor-inhibitor intervals accompanying either habituation or dishabituation (Fig. 7B), nor was there any significant change in the distribution of inhibitor-excitor intervals (Fig. 7C). The decrease in the number of 0–5 ms inhibitor-excitor intervals accompanying dishabituation is intriguing because that is the most critical interval for presynaptic inhibition (Marmont & Wiersma, 1938; Dudel & Kuffler, 1961*b*). We do not have enough data to say whether this change is reliable, however.

In summary, our data do not indicate that there was any consistent change in the pattern of evoked motor neurone activity which could account for the potentiation of claw opening following dishabituating stimulation.

Post-tetanic potentiation at the excitor neuromuscular synapse in the isolated claw preparation

A second possible explanation of the potentiation of claw opening following dishabituating stimulation is post-tetanic potentiation (PTP) at the excitor neuromuscular junction (Dudel & Kuffler, 1961*a*) or post-tetanic depression (PTD) at the inhibitor neuromuscular junction, since both the excitor and inhibitor usually fire during the dishabituating stimulation. In order to test these possibilities we performed experiments using the isolated claw preparation, where we could control the pattern of motor neurone activity by electrical stimulation of the excitor or inhibitor axons in the meropodite, and record excitatory junction potentials (EJPs) or inhibitory junction potentials (IJPs) with intracellular electrodes in the opener muscle.

PTP could be produced at the excitor neuromuscular junction with physiological parameters of stimulation. The standard protocol was to stimulate the excitor axon with a 'test' train of 30 Hz for 0·5 s, 30 s later deliver a 'conditioning' train of 10 Hz for 10 s, and 15 s after the onset of the conditioning train deliver a second test train. These parameters were all chosen to lie within the range of values encountered during dishabituation in the whole animal, such that the test train simulated the activity evoked by the habituating stimulus (tapping the thorax) and the conditioning train simulated the activity evoked by the dishabituating stimulus. EJPs were larger during the second test train (Figs. 8A, B) as a result of PTP produced by the conditioning train (if the conditioning train was omitted no increase in EJP

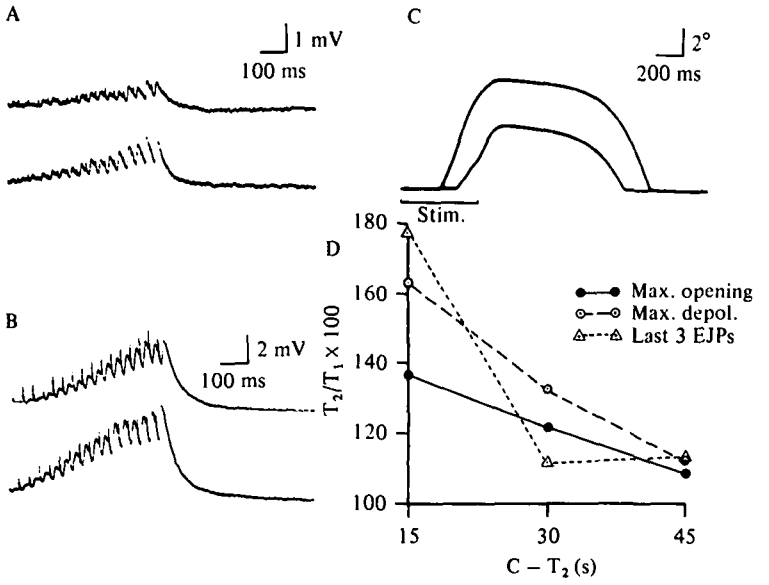

Fig. 8. PTP at the excitor neuromuscular junction. (A) EJPs recorded during test trains of excitor stimulation (30 Hz for 0·5 s) before (top trace) and 5 s after (bottom trace) a conditioning train of excitor stimulation (10 Hz for 10 s). (B) Records similar to those shown in part A, from another muscle fibre with larger EJPs. (C) Claw opening produced by test trains of excitor stimulation (62·5 Hz for 0·5 s) before and after a conditioning train of excitor stimulation (10 Hz for 10 s). (D) The average ratio of maximum opening (filled circles), maximum depolarization (open circles) or size of the last three EJPs (triangles) produced by the second (T_2) and first (T_1) test trains in experiments such as those shown in parts A, B and C at each of three conditioning-test (C-T_2) intervals (see text).

size was observed). PTP was observed in fibres from all regions of the muscle, regardless of the initial size of the EJPs. Potentiation of claw opening also occurs following conditioning stimulation in the isolated claw (Fig. 8C).

Two measures of PTP were computed: the ratio of the maximum depolarization during the second and first test trains and the ratio of the median of the last 3 EJPs. Fig. 8D shows the average values obtained for these measures in seven muscle fibres (from six preparations) at each of three conditioning-test intervals. A conditioning-test interval of 15 s simulates the first trial following the dishabituating stimulus, and a conditioning-test interval of 45 s simulates the second trial. This graph also shows the average potentiation of opening in five isolated claw preparations. For all three measures there was significant PTP overall ($F = 9{\cdot}74$ for the log of the ratio of median EJP's, 37·75 for maximum depolarization, and 83·0 for opening; $P < 0{\cdot}01$ in each case). For both maximum depolarization and opening there was also a significant effect of conditioning-test interval ($F = 5{\cdot}24$, $P < 0{\cdot}05$ and $F = 10{\cdot}42$, $P < 0{\cdot}01$, respectively). With a 15 s interval, PTP was significant for all three measures ($t = 3{\cdot}71$ for median EJPs, 5·27 for maximum depolarization, and 6·71 for opening, $P < 0{\cdot}01$ in each case), while with a 45 s interval it was not significant for any of the measures.

These results indicate that PTP occurring at the excitor neuromuscular junction as a result of activity evoked by the dishabituating stimulus can contribute significantly

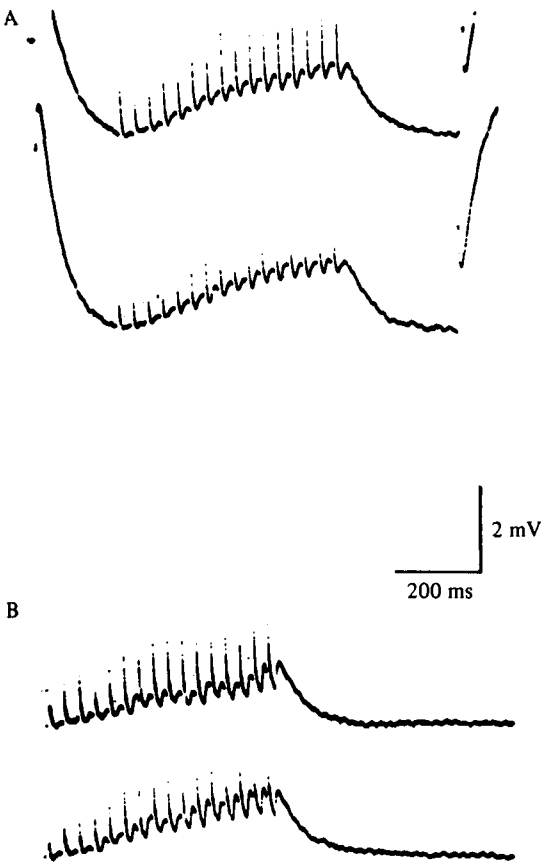

Fig. 9. Lack of PTP or PTD at the inhibitor junction. (A) IJPs recorded at a hyperpolarized level of membrane potential during test trains of inhibitor stimulation (30 Hz for 0·5 s) before (top trace) and after (bottom trace) a conditioning train of inhibitor stimulation (10 Hz for 10 s). (B) EJPs recorded during test trains of concurrent excitor and inhibitor stimulation (both 30 Hz for 0·5 s) before (top trace) and after (bottom trace) a conditioning train of inhibitor stimulation (10 Hz for 10 s).

to dishabituation, at least for one trial. Moreover, the magnitude of PTP is sufficient to quantitatively account for the potentiation of claw opening observed on the trial following dishabituation in the whole animal, which was 56% on the average (Fig. 6).

We next examined whether PTD occurs at the inhibitor junction in addition to the PTP observed at the excitor junction. An analogous protocol was used: we delivered a test train, 30 s later delivered a conditioning train of stimulation to the inhibitor axon, and 15 s later delivered a second test train. Test trains were of two types. Either we stimulated the inhibitor axon and measured the size of the IJPs at a hyperpolarized level of membrane potential (Fig. 9A), or we stimulated both the inhibitor and the excitor axons (with the inhibitor-excitor interval adjusted to produce partial but not complete inhibition) and measured the size of the EJPs (Fig. 9B). This second method is sensitive to changes in presynaptic as well as

postsynaptic inhibition. The parameters of the inhibitor trains were the same as have been used for the excitor (see above) and were within the range occurring during dishabituation in the whole animal. There was no evident change in the potentials produced by either type of test train as a result of the conditioning train (Figs. 9A, B). Similar negative results were obtained in each of three muscle fibres from two preparations. Since the same protocol revealed striking excitor PTP in every muscle fibre tested, we did not pursue these experiments further.

DISCUSSION

Motor neurone activity during claw opening

In agreement with previous studies (Wilson & Davis, 1965; Smith, 1975), we observed that tactile stimulation of the body produces co-activation of the opener excitor and inhibitor, with the excitor firing at a high rate initially and then gradually decelerating. Unlike studies which used more temporally diffuse stimulation such as brushing or scratching (Bush, 1962; Wilson & Davis, 1965; Smith, 1972, 1975), however, we observed a very regular pattern of inhibitor activity. This activity occurred in two bursts, an early and a late, separated by a period of inhibitor silence (and inferred inhibition of the inhibitor).

The function of the early inhibitor burst, which showed little variation from trial to trial, is not clear. It may serve as a 'clutch' permitting quick development of tension when the muscle is released from inhibition (Atwood, 1973), or it may be involved in co-ordination of the opener and stretcher, which share the same excitor motor neurone (Van Harreveld & Wiersma, 1937). The late inhibitor burst, which usually starts after movement has been initiated and is more variable, is probably involved in 'braking' the movement. Since it does not occur when the dactyl is heavily weighted (and hence braking is not necessary) it may depend on proprioceptive feedback, as previous authors have suggested (Bush, 1962; Wilson & Davis, 1965). On the other hand, the fact that this burst does not usually become weaker during habituation of claw opening argues against this idea and also against the possibility that it results from direct excitation of the inhibitor by the excitor (Wiens & Atwood, 1978).

Our algebraic model for the integration of excitor and inhibitor activity ($\theta = K_1 (E-K_2)/(I+K_3)$) differs from the traditional ratio model, which states that 'just complete inhibition' is achieved with a fixed ratio of steady-state inhibitor and excitor frequencies (Marmont & Wiersma, 1938). Smith (1975) extended the traditional model by proposing that the amplitude of evoked claw opening depends on the ratio of inhibitor to excitor frequencies, although his data are better described by our model (which would reduce to a simple ratio model if K_2 and K_3 were zero). Clearly a simple ratio model does not describe our data, since such a model would predict that a change from two to three inhibitor spikes, for example, should have drastic consequences, which is not the case (see Figs. 4 and 5). K_2 in our model probably represents the number of excitor spikes necessary to reach the threshold for excitation-contraction coupling. The physical interpretation of K_3 is not clear, but its mathematical interpretation is: for E greater than K_2, K_3 sets an upper limit on the opening achieved as I approaches zero.

Habituation of claw opening

Crayfish generally react to threatening stimuli with one of two rather stereotyped responses: defence, which consists of orienting towards the threat and raising and opening the claws, or escape, which consists of backwards swimming accomplished by a series of tail flips. Previous studies have shown that both the escape response (Krasne & Woodsmall, 1969; Wine, Krasne & Chen, 1975) and the defence response to mechanical (Hoffman, 1914) or visual (Glantz, 1974*a*) stimulation habituate. Interestingly, dishabituation of the escape response has not been demonstrated, whereas the defence response can be dishabituated by a variety of types of stimulation (Glantz, 1974*a* and this paper, Fig. 2).

Our results show that habituation of the claw opening component of the defence response is due to decreased firing of the opener excitor motor neurone, and not to increased firing of the inhibitor neurone as was previously suggested by Schöne (1961) on the basis of the results of Hoffmann (1914). The explanation of Hoffmann's results is not clear (unilateral sensitization is one possibility), but they do demonstrate the difficulties of interpreting lesion-type experiments. Several studies have shown that habituation of the crayfish escape response does not depend on inhibition (Krasne & Roberts, 1967; Zucker, 1972; Wine *et al.* 1975). In fact, in some cases inhibition acts to *prevent* habituation of that response (Bryan & Krasne, 1977). Rather, a primary role of inhibition in the escape response appears to be controlling the co-ordination and timing of the response (Wine, 1977), a role inhibition probably also plays in the defence response.

Habituation of the claw opening response is due not only to a decrease in the number of evoked excitor spikes, but also to an associated change in the distribution of excitor-excitor intervals (Fig. 7A). At the end of a series of trials there are fewer short intervals and thus less facilitation, so that each excitor spike produces less depolarization than at the beginning of a series (this effect can be seen in the EMG records in Figs. 4 and 5). Thus diminution of facilitation at the excitor neuromuscular junction may amplify the effect of reduction in the firing frequency of the motor neurone during habituation.

The mechanism producing a decrease in the firing frequency of the excitor motor neurone has not yet been investigated. Habituation of the defence response to visual stimulation and of the escape response to mechanical stimulation are evidently due to homosynaptic decrement at synapses from primary sensory neurones to sensory interneurones, which in turn make synaptic connexions onto command neurones (Glantz, 1974*b*, 1977; Krasne, 1969; Zucker, 1972). Since the response of mechanoreceptor interneurones decreases with repeated stimulation of the thorax (Arechiga *et al.* 1975), habituation of the defence response to mechanical stimulation may have a similar central mechanism.

Dishabituation of claw opening

Our results demonstrate three mechanisms of dishabituation of the crayfish claw opening response at the neuromuscular level: (1) increased firing of the opener excitor motor neurone, (2) decreased firing of the inhibitor motor neurone, and (3) PTP at the excitor neuromuscular junction. These three mechanisms can quanti-

tatively account for the average increase in claw opening on the trial following dishabituation (Figs. 6, 8).

Dishabituation is probably also due to increased excitation in spinal cat (Spencer *et al.* 1966) and in *Aplysia* (Castellucci *et al.* 1970). To our knowledge, this is the first demonstration that a change in firing of inhibitory neurones contributes to dishabituation. This result was somewhat surprising since inhibitor activity does not change during habituation of the claw opening response, suggesting that dishabituation of this response is not merely a reversal of the process of habituation (see also Groves & Thompson, 1970; Carew, Castellucci & Kandel, 1971). The mechanism of this effect is not known, but central inhibition of the inhibitor motor neurone is a possibility.

In addition to these central effects, potentiation at the periphery also contributes to dishabituation of the claw opening response. One probable mechanism of this potentiation is PTP at the excitor neuromuscular junction (Marmont & Wiersma, 1938; Dudel & Kuffler, 1961*a*). We have shown that PTP occurs with physiologically observed parameters of stimulation in the isolated claw and that the magnitude of PTP is sufficient to account for the potentiation of claw opening which occurs following dishabituation in the whole animal. Jacklet & Rine (1977) have made similar observations regarding dishabituation of the gill-withdrawal response in *Aplysia*, although their quantitative analysis was less complete, in part because they could not simultaneously record the activities of all the motor neurones involved. We observed no PTP or PTD at the opener inhibitor junction with physiological parameters of stimulation, even though facilitation of inhibition does occur (Marmont & Wiersma, 1938; Dudel & Kuffler, 1961*a*; this paper, Fig. 9A).

Another possible mechanism of potentiation of claw opening during dishabituation would be the release of a humoral factor such as octopamine or sertonin (Kravitz *et al.* 1976) in response to the arousal caused by the dishabituating stimulus. This mechanism could operate in addition to PTP (which is observed in isolated claw preparation where humoral factors are ruled out), although our ability to account quantitatively for the increase in claw opening following dishabituation suggests it is not important. Such a mechanism could also contribute to the potentiation occasionally observed on the second trial of a series, since the first trial would also be expected to produce arousal.

Our results indicate that changes both at the periphery and in the central nervous system contribute to dishabituation of the claw opening response. Dishabituation of the *Aplysia* gill-withdrawal response is similarly due to both an increase in the firing frequency of excitor motor neurones (Kupfermann *et al.* 1970) and PTP at excitor neuromuscular junctions (Jacklet & Rine, 1977). In *Aplysia*, increased firing of the motor neurones is due in part to heterosynaptic facilitation (Castellucci *et al.* 1970) and in part to PTP at central synapses (R. D. Hawkins, V. Castellucci and E. R. Kandel, *J. Neurophysiol.*, in press). In this paper we have analysed some peripheral mechanisms contributing to habituation and dishabituation of the crayfish claw opening response. The central mechanisms contributing to plasticity of this response remain to be investigated.

We would like to thank Dr Reid Hastie for his advice and assistance with the BMDPAR curve-fitting program. This research was supported in part by INSERM grant no. 7144443 to J.B. and by an NSF-CNRS postdoctoral fellowship to R.D.H.

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