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LONG-TERM ADAPTATION OF A PHASIC EXTENSOR MOTONEURONE IN CRAYFISH

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Summary

Long-term adaptation (LTA), a phenomenon previously studied in the crayfish claw, was examined in one of the motoneurones innervating the phasic abdominal extensor muscles. The motoneurone was conditioned by electrically stimulating the second root of the third abdominal ganglion *in situ* for 4 h per day, using trains of stimuli with an average impulse frequency of 2·5 Hz. In juvenile crayfish, 3 days of conditioning produced a marked (81 %) reduction in EPSP amplitude, which recovered only slightly during the succeeding 7 days. The quantal content of synaptic currents also decreased (by an average of 65 %). Estimated values of the binomial parameter *p* were lower for conditioned neurones than for controls, suggesting that the observed decrease in transmitter release involves a decrease in release probability. Conditioned neurones also displayed less synaptic depression than controls during repetitive stimulation at 5 Hz.

In adult crayfish, conditioning for 7 days also produced a marked (74 %) reduction in EPSP amplitude and resistance to synaptic depression. These results differ from those of previous work with the phasic axon of the claw closer muscle, which shows virtually no synaptic changes in adults after conditioning for 2 weeks. The ability to exhibit LTA, therefore, is not lost with age in all neurones.

Introduction

Experiments with the fast excitor neurone (FCE) supplying the claw closer muscle in juvenile crayfish have identified a distinct form of plasticity that persists for at least 1 week (Lnenicka & Atwood, 1985*a*,*b*). This phenomenon, known as long-term adaptation (LTA), is evoked by conditioning this relatively inactive neurone for 2 h per day for 3–14 days. Such conditioning causes the cell to release *less transmitter* at the beginning of a series of test stimuli and to display *less synaptic depression* when stimulated at 5 Hz. These changes make the FCE physiologically more similar to tonic crustacean neurones, which typically are more active, release less transmitter at low frequencies, and display marked facilitation but little depression (e.g. Atwood & Bittner, 1971). Conditioning also causes the synaptic terminals to become *more varicose* in appearance, and they can

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be seen to contain enlarged, branched mitochondria, making the phasic axon's terminals morphologically similar to those of tonic neurones (Lnenicka *et al.* 1986).

LTA appears to involve the neuronal cell body, since the synaptic changes can be prevented by severing the nerve containing the FCE axon (Lnenicka & Atwood, 1985*a*). In addition, LTA can be induced using procedures that preferentially depolarize the cell body and dendrites (Lnenicka & Atwood, 1985*a*,*b*). This suggests that the synaptic changes may involve macromolecules that are synthesized in the soma and transported down the axon. Further studies to identify such compounds are limited because the FCE axon and soma are not very accessible for amino acid incorporation studies and protein analysis. In addition, the synaptic changes exhibited by the FCE are restricted to juvenile crayfish, which would provide very little material for biochemical study.

The primary goal of the present study was to determine whether neurones other than the FCE exhibit LTA. We also hoped to identify other preparations that might be more suitable for examining the molecular mechanisms. We report here that conditioning stimuli supplied to an identifiable phasic abdominal extensor motoneurone elicit the electrophysiological changes characteristic of LTA: a long-lasting reduction in the initial amplitude of EPSPs and resistance to synaptic depression. In addition, these effects occur in both juvenile and adult crayfish, indicating that the ability of the extensor motoneurone to exhibit LTA is not lost with ageing.

Materials and methods

Animals

Crayfish (*Procambarus clarkii*), obtained commercially, were maintained in aerated freshwater tanks at 16°C on a diet of carrots and lentils. Most experiments were performed on 'intermediate-sized' juvenile crayfish (Lnenicka & Atwood, 1985*b*), which were 5–6 cm in total length (rostrum to telson), had carapace lengths ranging from 2·3 to 2·7 cm, and weighed approximately 3 g. In experiments involving adult crayfish, the animals were 10–13 cm long, had carapace lengths of 4·0–5·5 cm, and weighed between 14 and 45 g.

Conditioning

Extensor motoneurones were conditioned *in situ* by stimulating the second root of the third abdominal segment while monitoring the effects of stimulation by recording electromyographic potentials (EMGs) from the extensor muscles (Fig. 1). Stimulation was effected through two stainless-steel wires implanted through the dorsal surface of the abdomen on opposite sides of the second root (Fig. 1A). For EMG recordings, two wires were implanted near the posterior edge of the fourth abdominal segment, one lying near the midline and the other closer to the lateral edge. The wires were held in place with 'Five-minute Epoxy' and cyanoacrylate glues.

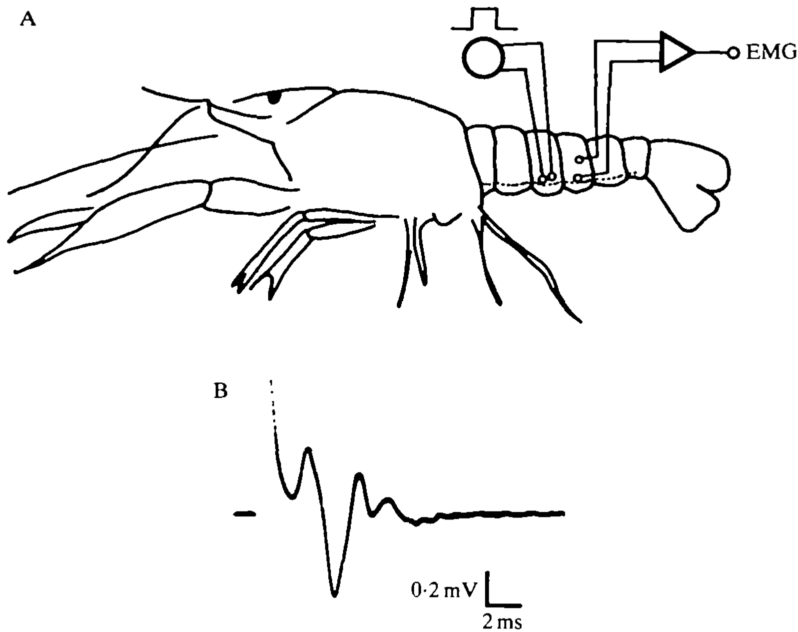

Fig. 1. Conditioning of extensor motoneurones *in situ*. Wires for stimulating the abdominal extensor motoneurones and for monitoring EMG activity in the extensor muscles were implanted in the positions indicated in A. A representative example of the complex EMG waveform elicited in response to maximal stimulation is shown in B. The complex EMG wave was produced by electrical activity in muscles located in both the third and fourth abdominal segments.

The second root of the third abdominal segment carries six motoneurones that innervate phasic extensor muscles in the third and fourth abdominal segments (Parnas & Atwood, 1966). The EMG electrodes recorded electrical activity from muscles in both segments. Thus, stimulating the extensor motoneurones produced a complex EMG waveform (Fig. 1B). The simplest procedure was to stimulate all the motoneurones during conditioning and, subsequently, to determine the effects of conditioning on a selected, identifiable neurone. Therefore, the intensity of the conditioning stimulus was kept just high enough to maintain the maximal EMG waveform. Subsequently, the synaptic properties of axon 3 (Parnas & Atwood, 1966) were studied (see below). A comparison of EMG recordings and intracellularly recorded EPSPs was made in three preparations and confirmed that the EMG wave resulted from activation of the phasic extensor neurones, including axon 3.

Conditioning stimuli were applied on the left side of the animal; homologous neurones on the right side served as controls. Responses to continuous stimulation at 5 Hz could not be maintained for long periods owing to depression, which caused the EMGs to diminish. Our aim was to observe responses throughout the conditioning period to ensure that the motoneurones had been activated and that the stimulating electrodes had not polarized. Depression was minimized by using

Table 1. *Composition of salines used in the present study*

	Concentration ($mmol\,l^{-1}$)	
Compound	5 × Mg saline	2·5 × Mg saline
NaCl	200·65	202·98
KCl	5·36	5·36
$CaCl_2.2H_2O$	6·75	10·12
$MgCl_2.6H_2O$	12·30	7·38
Hepes	1·00	1·00

(Modified from Van Harreveld, 1936).
In each case, the pH was adjusted to pH 7·4 with either HCl or NaOH.

1 s bursts of 5 Hz stimulation, delivered every 2 s, to give an average conditioning frequency of 2·5 Hz. Conditioning stimuli were applied for 4 h per day, to obtain a stimulus paradigm comparable to that employed for the FCE (Lnenicka & Atwood, 1985*a*,*b*).

Measurement of EPSPs and synaptic currents

The dorsal half of the abdomen was dissected as described by Parnas & Atwood (1966) and superfused with aerated saline. The temperature ranged from 13·5 to 15·5 °C but did not vary by more than 0·5 °C for any given preparation. It was necessary to lower the Ca^{2+} concentration and raise the level of Mg^{2+} in the saline to prevent rapid twitches that could dislodge the recording electrodes and damage the muscle cell under study. Two different salines were used (Table 1). One, which contained five times the normal concentration of Mg^{2+} (5 × Mg saline), greatly reduced the EPSP size and was used for most experiments. The other (2·5 × Mg saline) did not reduce the EPSPs as severely and was used mainly for studying synaptic depression. In both salines, the level of NaCl was adjusted to compensate for changes in osmolality.

Each segment of the abdomen contains three pairs of phasic extensor muscles, designated M, L_1 and L_2 (Parnas & Atwood, 1966). The present study took advantage of the fact that, of the six motor axons originating in the third abdominal segment, only one excitor (axon 3) and the common inhibitor (axon 5) innervate muscle L_1 of the fourth abdominal segment. EPSPs and synaptic currents were recorded from this muscle (Fig. 2) to simplify identification of the axon. In most experiments, axon 3 was selectively excited by placing a suction electrode with a very small tip diameter (20 μm) directly over it. With stimulation at 5 Hz, however, this procedure was not practical since the EPSPs initially facilitated, producing large contractions that dislodged the nerve from the stimulating electrode. In such cases it was necessary to stimulate the second root with a large suction electrode and to block the IPSPs from axon 5 using 1 $mmol\,l^{-1}$ picrotoxin (Atwood *et al.* 1967). Although the common inhibitor and all the

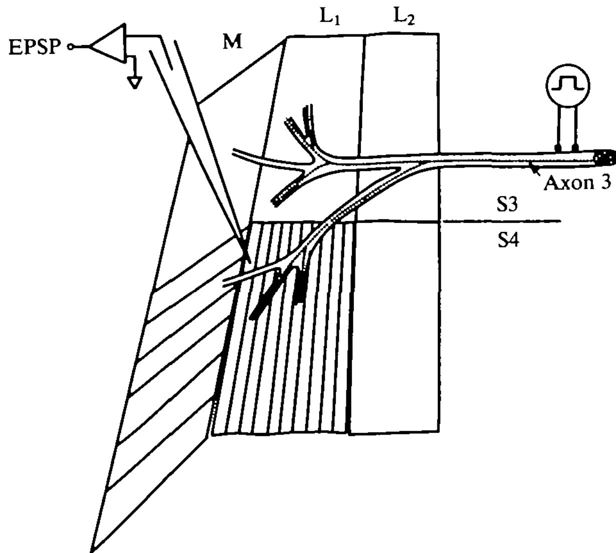

Fig. 2. Preparation for electrophysiological recording *in vitro*. The abdominal extensor muscles (L_1, L_2 and M) of the third and fourth abdominal segments (S3 and S4, respectively) are shown. (Only the left side of the animal is depicted, for simplicity.) Axon 3, which innervates muscles L_1 of the third and fourth segments, was stimulated using a suction electrode. All EPSPs and synaptic currents were recorded from muscle L_1 of the fourth segment. The drawing depicts an intracellular electrode inserted into the most medial muscle cell, which was easily discerned in all preparations.

excitatory neurones were probably driven during conditioning, we confined our analysis of synaptic properties to axon 3 and did not attempt to determine whether the other neurones were affected as well.

Intracellular recordings were obtained using conventional microelectrodes filled with $3\,\mathrm{mol\,l^{-1}}$ KCl and having resistances of 5–15 MΩ. All EPSP amplitudes were corrected for non-linear summation (Martin, 1955), assuming a reversal potential of +11·5 mV (Onodera & Takeuchi, 1975). Recordings were made from selected sites across the width of muscle L_1 with the aid of an ocular micrometer.

Quantal synaptic currents were recorded with 'macropatch' recording electrodes (Dudel, 1981) that had openings of 10 μm diameter at their tips. These electrodes were carefully positioned over synaptic spots, and the seal around the tip was improved by pressing gently against the surface of the muscle fibre. If there was any sign of damage, such as a high rate of spontaneous release, the recording site was not used. Recordings were stored on FM tape for subsequent analysis. Quantal content (m) was measured directly by counting the total number of quanta released and dividing by the total number of stimuli (Johnson & Wernig, 1971). A large number of stimuli (310–700) was supplied to the axon at a low frequency (0·5 Hz) which produced neither facilitation nor depression.

Binomial parameters p and n were estimated using a computerized method (B. Smith, J. M. Wojtowicz, and H. L. Atwood, in preparation; Wojtowicz *et al.*

1988), which: (a) generates various possible combinations of p and n, (b) calculates the 'maximum likelihood estimate' of n and p based on comparisons with the observed frequency distribution of quantal releases, and (c) selects the binomial values that provide the best fit to the observed distribution. A simple binomial model in which the probability of release is independent, stationary and uniform at all release sites was assumed. Since the model assumes stationariness of p, segments of the recorded responses (50 or 100 responses at a time) were analysed in series and, if the data showed a systematic change in p over time, the data were not used. Goodness of fit was determined by comparing the observed quantal distributions with those predicted from fitted values of p and n using a χ^2-test with $N-3$ degrees of freedom and employing Yates' correction factor (Johnson & Wernig, 1971). Only distributions having at least one degree of freedom with the χ^2-test (i.e. those with at least one release of three quanta) were considered for estimating the binomial parameters.

Statistical comparisons between the conditioned and contralateral (control) sides of the experimental animals were made using a t-test for matched pairs (Ferguson, 1971).

Results

EPSP amplitudes

Juvenile crayfish were conditioned for 3 days and were allowed to rest for 1 day before EPSPs were recorded in isolated preparations. EPSPs from axon 3 were recorded in $5\times$ Mg saline (Table 1) at a low stimulus frequency (0·1 Hz), which produced neither facilitation nor depression. Recordings were made at five positions, evenly spaced across the width of the muscle (Fig. 3). EPSP amplitudes from the conditioned side of the animal were significantly lower ($P<0{\cdot}005$) at all five recording sites. Combining the data from all sites gave mean values ($\pm$S.E.M.) of $1{\cdot}60\pm0{\cdot}47$ mV for the conditioned side and $8{\cdot}55\pm1{\cdot}44$ mV for the control side ($t=4{\cdot}529$; $P<0{\cdot}001$), for an average reduction of 81 %.

To investigate whether the reduction in EPSP amplitude was long-lasting, the time between the end of the 3-day conditioning procedure and EPSP measurement was varied from 1 to 7 days. EPSP amplitudes were measured at three sites in each muscle, and the results were averaged and expressed as a percentage of the mean EPSP amplitude on the unconditioned side (Fig. 4). During the first 5 days after conditioning, the EPSPs increased to about 50 % of control values, and there was no significant change thereafter. A linear regression of the data from which Fig. 4 was obtained gave a straight line with a slope significantly different from zero ($t=3{\cdot}38$; $N=31$; $P<0{\cdot}01$). This indicates a partial recovery of EPSP amplitude during the first 5 days after conditioning. The mean amplitude of EPSPs on day 7 was still significantly lower on the conditioned side ($4{\cdot}03\pm0{\cdot}48$ mV) than on the control side ($9{\cdot}49\pm1{\cdot}71$ mV; $N=7$; $t=3{\cdot}18$; $P<0{\cdot}01$). Thus, as with the FCE, the reduction in EPSP amplitude persisted for at least 1 week.

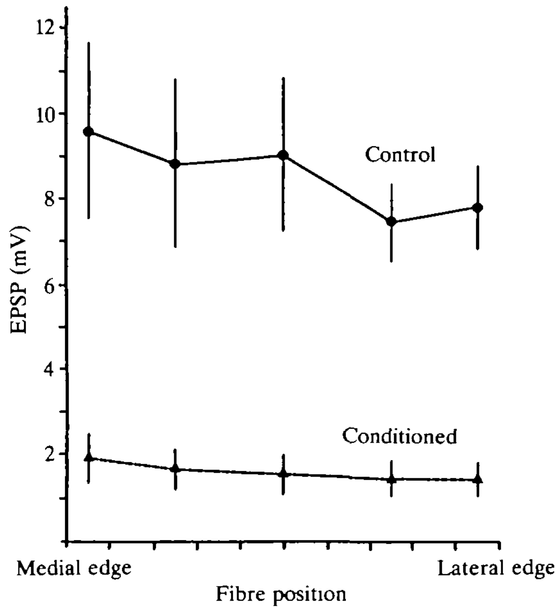

Fig. 3. Reduction in EPSP amplitude following 3 days of conditioning in juvenile crayfish. At each recording site, EPSP amplitudes were averaged from eight responses elicited at 0·1 Hz in each preparation. Results from 12 preparations were then averaged for each recording site. In this and all succeeding figures, error bars depict standard errors of the means.

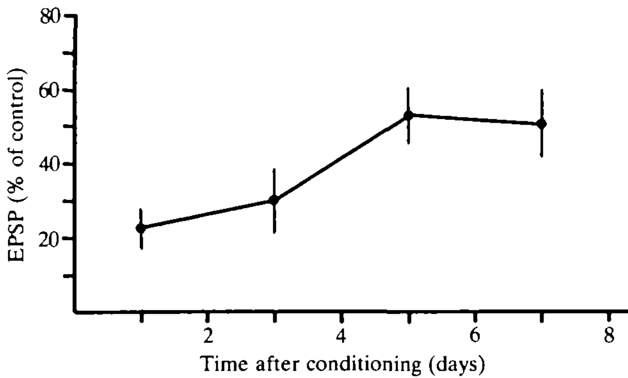

Fig. 4. Partial recovery of EPSPs. Juvenile crayfish were conditioned for 3 days, and EPSP amplitudes were measured at various times thereafter. In each muscle, EPSPs were recorded in the medial and lateral muscle cells and at one site half-way between. EPSP amplitudes at each site were averaged from eight responses at 0·1 Hz, and the three sites were combined to give an average value for the muscle. Values from each conditioned muscle were then expressed as a percentage of the contralateral control. The data represent mean values from 12, 6, 6 and 7 preparations measured on days 1, 3, 5 and 7, respectively. A linear regression of the raw data gave a linear equation ($y = 5{\cdot}26x + 17{\cdot}5$), not illustrated.

Depression at 5 Hz

The effect of conditioning on neuromuscular depression was examined by comparing initial values of the EPSP amplitude (measured at 0·1 Hz) with values obtained at the end of 20 min of continuous stimulation at 5 Hz. The 5 × Mg saline caused a very large reduction in the amplitude of unfacilitated EPSPs and significant facilitation at 5 Hz, which masked any depression for 20 min or longer. To study depression, a saline representing a more modest reduction in the ratio of Ca^{2+} to Mg^{2+} was used (2·5× Mg saline). This did not reduce the amplitude of unfacilitated EPSPs as severely (compare Figs 3 and 5) and allowed depression to occur within 20 min at 5 Hz. Facilitation was observed at 5 Hz and usually led to regenerative responses and rapid twitches, which were particularly prominent in unconditioned muscles. To avoid muscle damage, the microelectrode was removed after responses had been recorded at low frequency (0·1 Hz) and subsequently was re-inserted into the same cell after contractions at 5 Hz had subsided. EPSPs were obtained only from the muscle cell at the medial edge of L_1, since this cell was clearly visible in all preparations and could be reliably identified and penetrated. Responses at 5 Hz were monitored throughout using an extracellular electrode to record field potentials.

The amplitudes of EPSPs recorded before and at the end of 20 min of stimulation at 5 Hz are shown in Fig. 5A, which was obtained from animals that had been conditioned for 3 days and allowed 3–4 days to rest. At the end of the 5 Hz train, EPSPs on the control side had decreased by 94 % of their initial, unfacilitated amplitude (measured at 0·1 Hz), whereas only a 35 % reduction had

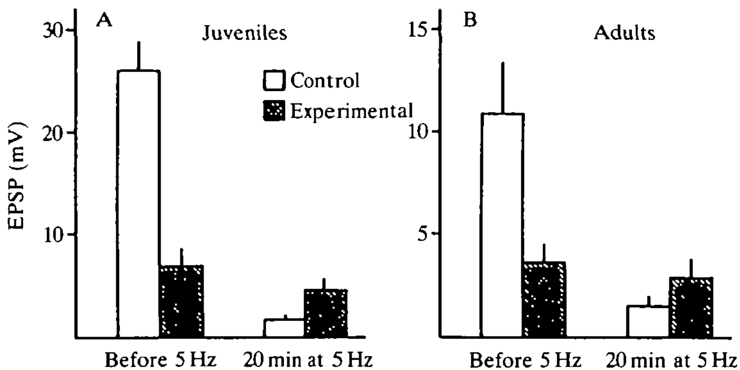

Fig. 5. Resistance to synaptic depression. EPSPs were recorded from the muscle cell lying on the medial edge of muscle L_1 (see Fig. 2). Initial EPSP amplitudes were measured before the application of a 5 Hz train by averaging eight responses at 0·1 Hz. The axon was then stimulated at 5 Hz for 20 min, and EPSP amplitudes at the end of the train were obtained by averaging 16 responses (at 5 Hz). Results shown in A were obtained from juvenile crayfish ($N = 8$) that had been conditioned for 3 days. Results shown in B were obtained from adult crayfish ($N = 6$) that had been conditioned for 7 days. In both cases, the animals were allowed to rest for 3–4 days before EPSP amplitudes were measured.

occurred on the conditioned side (Fig. 5). This resulted in a mean EPSP amplitude that was significantly higher on the conditioned side ($t = 2{\cdot}64$; $N = 8$ preparations; $P < 0{\cdot}025$). Thus, as reported for the FCE (Lnenicka & Atwood, 1985*b*), conditioning produced a smaller initial EPSP that was more resistant to depression at 5 Hz.

LTA in adult crayfish

Adult crayfish, weighing up to 45 g, were conditioned in the same manner as juvenile crayfish, except that the conditioning period was extended from 3 to 7 days. EPSPs were recorded 3–4 days after the end of conditoning. EPSP amplitudes, averaged from three sites on each muscle, were lower for conditioned neurones ($3{\cdot}69 \pm 0{\cdot}71$ mV) than for controls ($14{\cdot}2 \pm 2{\cdot}2$ mV), and the differences were statistically significant ($t = 4{\cdot}48$; $N = 9$; $P < 0{\cdot}005$). Overall, a 74 % reduction in EPSP amplitude was observed. Synaptic depression, which was examined in six preparations, showed the same trend as for juvenile crayfish (Fig. 5B). Stimulation at 5 Hz produced a small (21 %) reduction in EPSP amplitude on the conditioned side and a large (86 %) reduction on the control side. At the end of the 5 Hz train, EPSP amplitudes were higher on the conditioned side than on the control side ($t = 2{\cdot}71$; $P < 0{\cdot}025$).

Quantal content

Synaptic currents were recorded in juvenile crayfish (in 5×Mg saline) to determine whether the reduction in EPSP amplitude reflected a change in transmitter release. Individual quanta, indicated by distinct steps in the current trace (Fig. 6A), were clearly distinguishable. The quantal currents were approximately 100–500 pA in amplitude and had rise times of less than 0·5 ms. Quantal content (m) was estimated at each recording site by applying up to 700 stimuli to the axon, counting the number of occurrences of 0, 1, 2, 3 or more quantal releases (e.g. Fig. 6B), and dividing the total number of releases by the number of stimuli (Johnson & Wernig, 1971). Recordings were made from one, two or three sites on a given muscle, and the quantal contents were combined to give an average value of m for that preparation. In all, 11 sites from conditioned extensors and 10 sites from their contralateral controls were examined. Mean values of m (Fig. 6C) were significantly smaller on the conditioned side ($t = 3{\cdot}818$; $N = 7$ preparations; $P < 0{\cdot}005$), and indicated an average reduction in quantal content of 65 %.

Binomial parameters n and p were estimated for recording sites that met the following criteria: (a) the rate of release did not vary consistently over time and (b) the number of releases was sufficient to compare the observed and predicted frequency distributions using a χ^2-test with N−3 degrees of freedom (see Materials and methods). Ten recording sites (five conditioned and five controls) from four animals met both criteria (Table 2). These sites showed a decrease in the estimated value of p in each preparation. In most cases, the estimated value of p was very low on the conditioned side (<0·10), making estimates of n highly unreliable

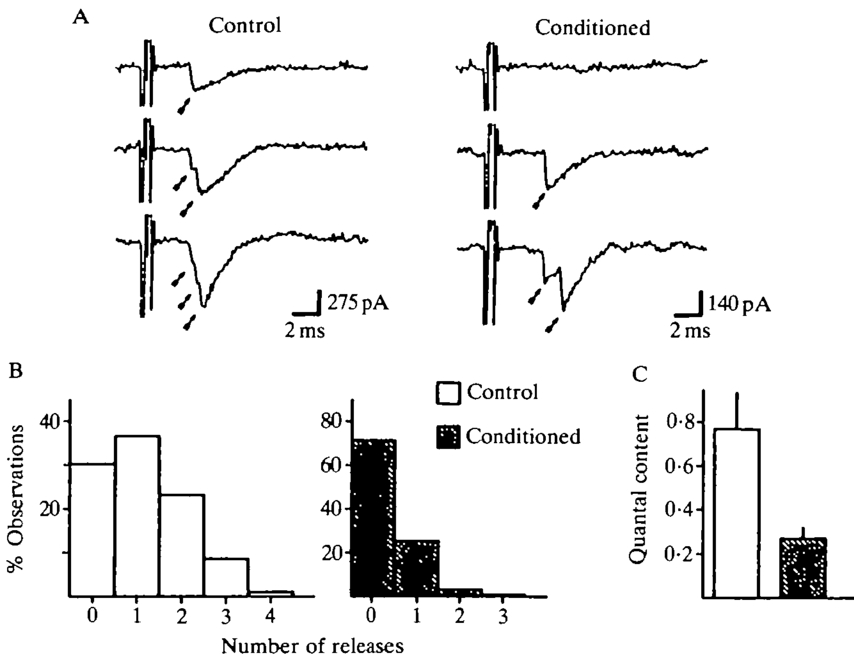

Fig. 6. Quantal currents and quantal content. Extracellularly recorded synaptic currents in a control muscle and in a conditioned muscle from the same animal are shown in A. (Arrows indicate individual quanta that were counted for analysis.) B depicts the frequency distributions of observed releases of 0, 1, 2, 3 or more quanta, obtained from the two recording sites represented in A. The mean quantal content was 1·13 for the control site and 0·34 for the conditioned site. (The total numbers of stimuli were 631 and 690, respectively.) C shows mean quantal contents measured in seven animals. Quantal content was measured at one, two or three sites in any given muscle, and the results were combined to obtain an average value for that muscle. The histograms depict mean values (+ standard errors) for seven conditioned muscles and for their contralateral controls. Results in A, B and C are from juvenile crayfish that had been conditioned for 3 days and allowed 1 day to rest.

(McLachlan, 1978). Therefore, no conclusions are drawn regarding the question of whether changes in n also occurred.

Discussion

The present study demonstrates LTA in one of the phasic motoneurones innervating the crayfish abdominal extensor muscles. The electrophysiological changes induced by daily conditioning are similar in most respects to those previously reported for the crayfish FCE. Such similarities include a marked reduction in transmitter release, a decrease in synaptic depression at 5 Hz, and a long persistence (at least 7 days). Unlike the FCE, however, the extensor neurone investigated in the present study does not lose the ability to exhibit LTA during the transition to adulthood.

Table 2. *Binomial analysis of recorded quantal currents*

	Control				Conditioned			
Expt	m	*p*	*n*	$P(\chi^2)$	m	*p*	*n*	$P(\chi^2)$
1	2·08	0·45	5	>0·1	0·59	0·20	3	>0·1
	1·13	0·16	7	>0·1	0·33	0·05	*	>0·3
2	1·09	0·24	4	>0·5	0·32	0·02	*	>0·2
3	0·68	0·23	3	>0·5	0·35	0·12	3	>0·8
4	0·85	0·28	3	>0·1	0·19	0·02	*	>0·3

Data from individual recording sites in four different animals are shown.
Values indicate mean quantal content (m), estimated binomial parameters (*n* and *p*), and the significance level of the χ^2-test used in comparing the observed frequency distribution of quantal currents with the predicted distribution [$P(\chi^2)$].
A value of $P(\chi^2)$ of less than 0·05 would indicate that the observed and predicted frequency distributions are significantly different.
In no case was there any significant difference between the observed distribution and the distribution predicted from estimated values of *n* and *p*.
* Values for *n* are reported only for $P > 0{\cdot}10$.

The synaptic current recordings provide direct evidence that conditioning causes a reduction in transmitter release. A quantal analysis of initial EPSPs was not possible with the FCE, which displays marked synaptic depression at low stimulus frequencies (Pahapill *et al.* 1985). However, differences in quantal content between conditioned and unconditioned FCE axons were apparent at the end of a 5 Hz train (Lnenicka & Atwood, 1985*b*). The cumulative evidence from both systems demonstrates directly that conditioning causes the nerve terminals to release less transmitter initially, whereas at the end of a high-frequency train the amount of transmitter released is actually greater for conditioned axons than for controls.

Possible changes in input resistance of the postsynaptic muscle cells were not investigated in the present study. The observed decrease in quantal content (65 %), however, was large enough to account for most of the observed reduction in EPSP amplitudes (81 %). We did not attempt to determine whether conditioning caused a change in mean quantal size, since the amplitude of individual quantal events at each recording site depends on the seal resistance around the rim of the macropatch electrode, which might not be the same in each case. Conditioning the FCE causes no change in input resistance of its postsynaptic muscle fibres (Lnenicka & Atwood, 1985*b*).

Binomial analysis indicates that the decreased quantal content following conditioning involves a reduction in estimated values of the binomial parameter *p*. This suggests a decrease in the mean probability of transmitter release (Atwood & Wojtowicz, 1986; Korn *et al.* 1982). The parameter *n* could not be reliably estimated for the majority of recording sites on conditioned muscles because the

estimated value for p was small. This stems from the fact that n is estimated from the relationship:

$$n^* = m/p^*, \quad (1)$$

where n^* and p^* represent estimated values for n and p, respectively. When p is very small, even small errors in the estimate of p can produce very large errors in estimating n (McLachlan, 1978).

The binomial analysis performed in the present study assumed a simple binomial model in which the probability of release at each site is equal and independent. Although this may not always be the case at crustacean neuromuscular synapses (Atwood & Tse, 1988; B. Smith, J. M. Wojtowicz & H. L. Atwood, in preparation), there was not sufficient evidence to reject the simple binomial model for any of the quantal distributions reported in the present study, as indicated by the χ^2-test.

A reduction in transmitter release can result from a decrease in the pool of transmitter immediately available for release or from changes in the ability of the nerve terminal to activate transmitter release. Cumulative evidence favours the latter process as a mechanism underlying LTA. Conditioning does not appear to deplete the stores of available transmitter, since conditioned neurones still display facilitation and even release more transmitter than controls when stimulated continuously at 5 Hz (Lnenicka & Atwood, 1985*b*). A similar argument has been made for the frog cutaneous pectoris preparation (Hinz & Wernig, 1988), where the degree of facilitation is greater for conditioned axons than for controls. The decrease in the binomial parameter p observed in the present study suggests a decrease in the probability of transmitter release at individual release sites and, thus, provides some evidence for a direct effect on excitation–secretion coupling.

The reduction in quantal content during LTA is likely to involve mechanisms that decrease calcium influx into the nerve terminals. Such mechanisms could include decreased depolarization in the synaptic regions (Parnas *et al.* 1982), possibly through a loss of electrical excitability of the nerve terminals (Dudel *et al.* 1984). Alternatively, the calcium channels could be affected directly.

The ability of the conditioned nerve to sustain transmitter release at 5 Hz is thought to be related to observed increases in mitochondrial volume within the synaptic terminals of the FCE (Lnenicka *et al.* 1986). It remains to be shown whether such changes also accompany LTA in the abdominal extensors. Further studies of the time course of such changes are also warranted.

The appearance of LTA in adult crayfish is somewhat surprising. Previous experiments with the FCE showed that conditioning for up to 14 days caused no change in initial EPSP amplitude and only small changes in fatigue resistance compared with pronounced changes in these factors in juvenile crayfish (Lnenicka & Atwood, 1985*b*). Such age-dependence was thought to reflect a greater potential for synaptic plasticity in younger animals, presumably because greater synaptic growth may be required to compensate for changes in postsynaptic input resistance as the muscle fibres grow (Lnenicka & Mellon, 1983). In the present

study, a longer conditioning period was used for adult animals than for juveniles (7 days instead of 3 days). Thus, it is not clear whether older animals display LTA as readily as do juveniles. Nevertheless, the data indicate that at least some neurones do not lose the ability to exhibit LTA during ageing.

To date, experiments aimed at studying LTA have been limited to crayfish motoneurones. However, a recent study involving the motoneurones that innervate frog cutaneous pectoris muscles (Hinz & Wernig, 1988) demonstrates a phenomenon very similar to LTA. Stimulation of the motoneurones for several hours per day for 5–8 days caused reductions in evoked endplate potentials and in quantal content similar in magnitude to the changes reported here. In addition, the degree of synaptic depression during brief, high-frequency trains was reduced (Hinz & Wernig, 1988).

Evidence from a number of systems indicates that chronic decreases in activity also produce synaptic changes consistent with LTA. For example, tetrodotoxin blockade increases the amplitude of EPSPs generated by guinea-pig sympathetic neurones (Gallego & Geijo, 1987), by Ia sensory afferent neurones (Gallego *et al.* 1979) and by mammalian motoneurones (Snider & Harris, 1979). Immobilization causes increased transmitter release and increased synaptic fatigue in vertebrate motoneurones (Robbins & Fischbach, 1971) and in the crayfish FCE (Pahapill *et al.* 1985). Sensory deprivation causes similar changes in the developing sensory systems of insects (Bloom & Atwood, 1980; Murphey & Matsumoto, 1976). Thus, a growing body of evidence suggests that LTA reflects a general property of neurones, allowing them to adjust their release characteristics to suit the demands of their chronic activity patterns.

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