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Review

Synaptic diversity and differentiation: crustacean neuromuscular junctions

H. L. ATWOOD and R. L. COOPER

Department of Physiology, Medical Sciences Building, MRC Neural Group, University of Toronto, Toronto, Ontario, Canada M5S 1A8

ABSTRACT Crustacean motor neurons exhibit a wide range of synaptic responses. Tonically active neurons generally produce small excitatory postsynaptic potentials (EPSPs) at low impulse frequencies, and are able to release much more transmitter as the impulse frequency increases. Phasic neurons typically generate large EPSPs in their target cells, but have less capability for frequency facilitation, and undergo synaptic depression during maintained activity. These differences depend in part upon the neuron's ongoing levels of activity; phasic neurons acquire physiological and morphological features of tonic neurons when their activity level is altered. Molecules responsible for adaptation to activity can be sought in single identified phasic neurons with current techniques. The fact that both phasic and tonic neurons innervate the same target muscle fibers is evidence for presynaptic determination of synaptic properties, but there is also evidence for postsynaptic determination of specific properties of different endings of a single neuron. The occurrence of high- and low-output endings of the same tonic motor neurons on different muscle fibers suggests a target-specific influence on synaptic properties. Structural variation of synapses on individual terminal varicosities leads to the hypothesis that individual synapses have different probabilities for release of transmitter. We hypothesize that structurally complex synapses have a higher probability for release than the less complex synapses. This provides an explanation for the larger quantal contents of 'high-output' terminals (where the proportion of complex synapses is higher), and also a mechanism for progressive recruitment of synapses during frequency facilitation.

KEY WORDS: presynaptic; crustacean; synapse; plasticity; ultrastructure; adaptation

Introduction

The crustacean neuromuscular junction has long served as a valuable model for studies of synaptic transmission, and there is good reason to believe that it will continue to do so. The advantages offered by crustacean motor neurons are several:

(1) Identifiable neurons. Each muscle is innervated by a small number of distinct motor neurons, each of which can be uniquely identified both centrally and peripherally. For experimental work, one can be certain of always selecting a known neuron. This advantage is not limited to crustaceans, as insects have a similar organization of the motor systems. Giant central neurons of *Aplysia*, squid, and other molluscan species can also be uniquely identified, as can Mauthner cells of fish.

(2) Large size. The peripheral motor axons of crustaceans are among the largest conveniently available, and the centrally located cell bodies of these neurons are also large. It is possible to penetrate peripherally located axon processes with electrodes and inject various materials into them, including calcium indicators, toxins, and other pharmacological agents which act on synaptic transmission. Here also, the advantage of size is not confined to crustacean neurons, since some molluscan central neurons are larger, as is the squid giant synapse.

(3) Central-type synaptic physiology in the periphery. This feature gives the crustacean motor system a continuing attractiveness. Properties of short-term and long-term synaptic plasticity as well as inhibitory pre- and post-synaptic mechanisms are strongly expressed in crustacean peripheral synapses and definitely more so than in the squid giant synapse or vertebrate neuromuscular junctions, which are more specialized for effective triggering of action potentials in their postsynaptic target cells, and in which features such as short-term facilitation cannot normally be seen unless transmission is artificially depressed.

(4) Easily observed morphology and ultrastructure. Use of fluorescent vital dyes enables the experimenter to match physiological measurements with a readily observable nerve process. Further work on the same structure at the electron microscopic level provides information on occurrence and structure of the synapses which generate the physiological responses under investigation.

(5) Long life span of individual neurons. This feature has permitted sequential studies of the same neuron over long periods of time (many years, in the

Corresponding author: Dr H. L. Atwood

case of the long-lived American lobster). Although not fully exploited for studies of neuronal aging, regeneration, synapse turnover, and trophic interactions between the neuron and its target cells, there is nevertheless good potential for such work.

A drawback, at present, of crustacean motor systems for investigation of synapses is the lack of information at the molecular level on specific proteins involved in neuronal function. Most of the recent information on specific molecules has been obtained in other species, including mammalian species and *Drosophila*. The relative phylogenetic closeness of crustaceans and *Drosophila* offers the possibility that crustacean synaptic molecules will eventually become better known through their similarity to counterparts in *Drosophila*, but this avenue of approach is only beginning to be explored.

In the present review, we address recent work on synaptic differentiation and plasticity in crustacean synapses. For this purpose, we compare physiological and structural observations of 'phasic' and 'tonic' motor neurons, and of physiologically different endings of a single motor neuron innervating different target cells. We discuss the extent to which structural observations account for the functional differences, and possible pre- and post-synaptic determinants of synaptic properties. We also present selected features of short- and long-term synaptic plasticity, including a preliminary approach to altered gene expression involved in long-term neuronal adaptation.

Features of innervation

Invertebrate muscle is innervated differently from vertebrate skeletal muscle. Invertebrate muscle fibers are commonly multiterminally innervated, and can also be polyneuronally innervated by excitatory as well as inhibitory motor neurons (Atwood, 1972, 1973a, b, 1976). For addressing the mechanisms for the differences in structure and physiology of presynaptic terminals, crustacean muscles offer several advantages, including: (1) preparations with two different neurons innervating the same target, which provide an opportunity to examine intrinsic neuronal differences; and (2) preparations in which a single neuron innervates two different sets of target cells, which provide an opportunity to assess mechanisms of synaptic differentiation, including retrograde signalling. Examples of these two types of preparations are taken from the freshwater crayfish (Figs 1 and 2).

The extensor muscle in the walking legs of crayfish offers the advantage of two motor neurons, one phasic and one tonic, that innervate the same muscle fibers. The general innervation pattern of the extensor muscle reveals two structurally distinct motor nerve terminal types (Fig. $1A_1$). The tonic motor nerve terminal has pronounced varicosities along its length, and the diameter of these varicosities can vary enormously along the terminal length, particularly in adult crayfish. The phasic motor nerve terminals, in contrast, are thin and filiform with very small varicosities appearing

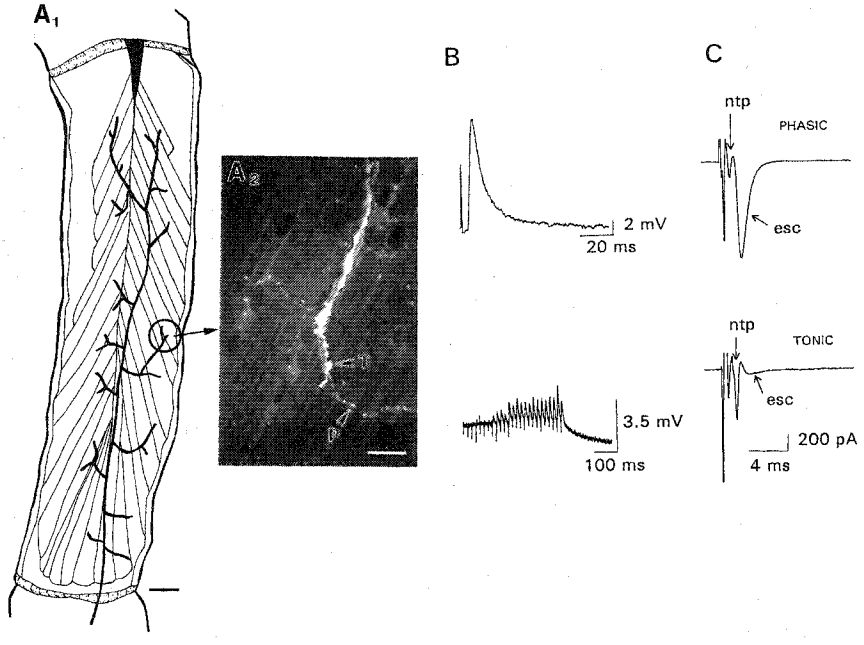

Fig. 1. Differential morphology and physiology of two excitatory motor neurons supplying the same set of postsynaptic muscle fibers (the extensor muscle of the carpopodite in the crayfish leg). A. *The general distribution of the innervation (A_1) and a representative region of innervation (A_2) are illustrated. In the latter, thin phasic terminals with small varicosities (P), and a larger tonic terminal with pronounced varicosities (T) are distributed to the underlying muscle fiber. B, C. Representative intracellular (B) and extracellular synaptic (C) recordings during selective stimulation of phasic (upper records) and tonic (lower records) excitatory motor axons. B. Single stimuli to the phasic axon elicit EPSPs of 10–15 mV throughout the muscle, whereas EPSPs of the tonic axon are negligible at 1Hz and develop appreciable amplitude only at frequencies greater than 5 Hz. The trace shown is at 60 Hz. C. Synaptic currents recorded with a macro-patch electrode placed over individual phasic and tonic varicosities, to show the difference in averaged evoked response (ntp, extracellular nerve terminal potential; esc, averaged excitatory synaptic current). With a recording electrode of 10–15 μm diameter, quantal contents at 1 Hz are typically very low (0.1) for the tonic axon, and high (10) for the phasic axon. Scale bar: A_1 500 μm; A_2 25 μm.*

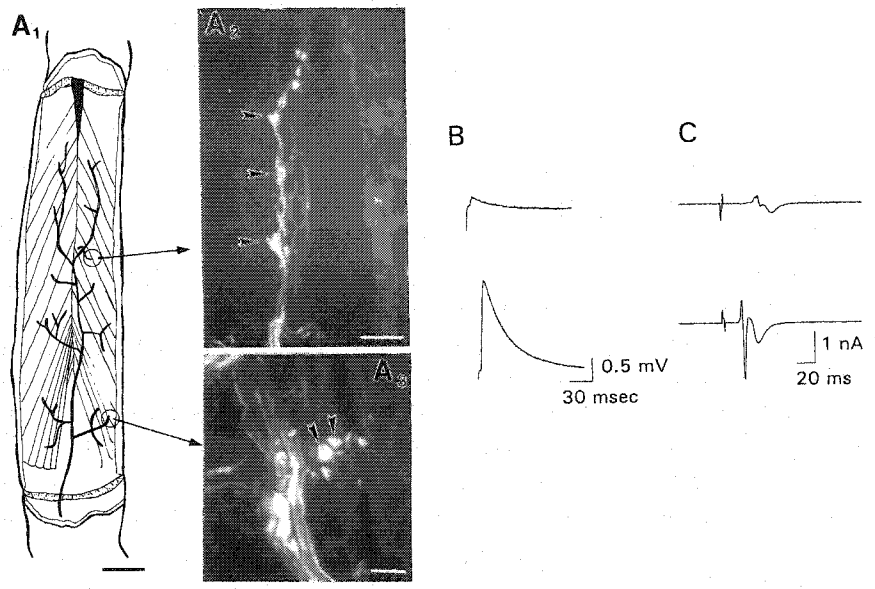

Fig. 2. Differential morphology and physiology of endings from a single excitatory axon supplying two regions of the crayfish opener muscle. A. *The general distribution of the innervation over the inner surface of the opener muscle (A_1), and representative terminals stained with 4-Di-2-Asp in central (A_2) and proximal (A_3) regions of the muscle. Two axons, the opener excitor axon and the opener inhibitor axon, run closely together and have varicosities (arrow heads) of similar size. Central terminals are relatively long, with varicosities (arrow heads) arranged in series, while proximal terminals are often shorter, with varicosities arranged in clusters.* B. *Intracellularly recorded EPSPs (averaged at 1 Hz stimulation) for central (upper) and proximal (lower) muscle fibers, showing an 8.5-fold difference in amplitude in this example.* C. *Extracelluarly recorded synaptic responses from central (upper) and proximal (lower) varicosities averaged at 1 Hz, to illustrate the larger response (due to higher quantal output) in the proximal varicosities. Scale bars: A_1 500 μm; A_2 25 μm; A_3 10 μm.*

periodically along the terminal (Fig. $1A_2$).

For investigating synaptic differentiation of a single neuron, the opener muscle preparation from the legs of crayfish is quite suitable (Fig. $2A_1$). A single excitatory motor neuron innervates the entire muscle, but the EPSPs for fibers at different locations can vary as much as 8-fold (Iravani, 1965; Cooper *et al.*, 1995b). In vital, dye-stained preparations, morphological correlates of terminal differences can be discerned. The terminals in the proximal region, where the larger EPSPs are recorded, are generally shorter than those in the central region, and often produce clusters of varicosities near the main axon branches (Fig. $2A_3$). Quantification of these differences was achieved by comparing terminal branches and numbers of varicosities in the two regions, and it was found that there are fewer varicosities per muscle fiber in the proximal region than in the central region, where the fibers display smaller EPSPs.

Synaptic physiology

Types of motor neuron

Excitatory motor neurons supplying many crustacean limb muscles were classed as 'fast' or 'slow' by Wiersma and co-workers (Wiersma, 1961), according to the type of muscle contraction they evoked when stimulated. Later work on abdominal muscles of the crayfish (Kennedy and Takeda, 1965a, b; Parnas and Atwood, 1966) established a general phasic/tonic dichotomy for excitatory motor neurons. 'Phasic' neurons supplying the fast-acting (twitch) muscles of the crayfish abdomen are generally silent, being recruited only for swimming (escape) responses. They produce large EPSPs in their target muscles, and these EPSPs often evoke muscle action potentials accompanied by twitch contractions. However, repetitive stimulation generally leads to rapid depression of the EPSP. These physiological effects are attributable to initial large output of transmitter from innervating nerve terminals, and its subsequent decline. In contrast, 'tonic' neurons supplying slow-acting, postural muscles of the abdomen are usually active, adjusting postural responses continuously. Synaptic transmission is not readily depressed in these neurons, even with maintained high-frequency stimulation. Most of the same distinctions apply to the 'fast' and 'slow' motor neurons of limb muscles (Hoyle and Wiersma, 1958), which can also be referred to as 'phasic' and 'tonic', respectively, on the basis of their activity patterns (Atwood and Walcott, 1965).

Among the limb muscles, some (such as the crayfish opener muscle) receive only a single excitatory motor axon with tonic properties, while others (such as the crayfish claw closer and the main limb extensor) receive both phasic and tonic axons. In the latter muscles, the majority of the muscle fibers are innervated by both phasic and tonic axons, but there is often physiological and/or biochemical differentiation of the muscle fibers, with the rapidly contracting fibers receiving particularly strong input from the 'phasic' axon, and the more slowly contracting fibers receiving a strong 'tonic' input (Atwood, 1965; Atwood and Hoyle, 1965). Such differential input, combined with

the differences in synaptic physiology, provides the basis for the 'fast' and 'slow' contractions of a single muscle originally described by Wiersma and co-workers (Wiersma, 1961).

Analysis of the fundamental reasons for the synaptic differences in phasic and tonic motor neurons is far from complete. Local stimulation of nerve endings has indicated that phasic terminals appear to be excitable (Dudel *et al.*, 1984), whereas some tonic terminals may not be fully excitable (Dudel, 1982; Parnas *et al.*, 1984). It has never been well established whether or not the varicose terminals of tonic axons conduct an action potential over their entire length; conflicting views are found in studies to date (Zucker, 1974a, b; Dudel, 1982). Thus, the basis for the relatively large initial release of transmitter by the phasic terminals may be due in part to electrical differences of the terminal, including differences in amplitude or duration of the action potential. Preliminary ultrastructural work on synapses of the crayfish leg extensor muscle clearly indicates that for a given length of terminal, there are more individual synapses on the tonic than on the phasic terminal, even though quantal release of transmitter is much greater for a single impulse in the phasic terminal (M. J. King, H. L. Atwood and C. K. Govind, unpublished observations). The number of synapses per length of terminal cannot account for the higher output of transmitter, and the probability of transmitter release per synapse is considerably higher for phasic terminals. Several factors could contribute to this situation, including electrical differences alluded to above, or molecular differences concerned with regulation of transmitter release (Atwood *et al.*, 1995).

With respect to the more rapid depression of the phasic terminals, differences in mitochondrial content (Lnenicka *et al.*, 1986) and metabolic capability (Nguyen and Atwood, 1992a, 1994) are likely to be involved. Maintaining the transmitter supply and vesicle recycling requires energy, and the tonic terminals appear to be much more adapted to meet energy demands of a continuous nature. Furthermore, glutamate levels are higher in tonic terminals (Shupliakov *et al.*, 1995) thereby providing the possibility for more rapid replenishment of vesicles with transmitter. The role of mitochondria in phasic–tonic differentiation is also supported by mitochondrial changes during conversion of phasic axons to a more tonic phenotype with imposed additional activity (Lnenicka and Atwood, 1985b; Nguyen and Atwood, 1994).

Differentiation of nerve terminals of a single motor neuron

Physiological differences in the EPSPs set up by a single motor neuron in different target muscle fibers are commonly observed in crustacean limb muscles (Atwood, 1967; Bittner, 1968). Most of the observations have been gathered from limb muscles with a single (tonic) excitatory motor axon. With low-frequency stimulation, a range of EPSP amplitudes is observed; in the crayfish leg opener muscle, for example, the large EPSPs in the proximal bundles of the muscle are 8–10 times larger than the smaller EPSPs in the central part of the muscle (Iravani, 1965; Cooper *et al.*, 1995b). Analysis of the reasons for this has indicated that about 1/4 of the difference is due to the higher input resistance of the proximal muscle fibers, and the rest to other factors, the most important being the higher quantal release per impulse from varicosities in the proximal region (Cooper *et al.*, 1995b). Since the number of synapses per varicosity is about the same for the two regions (Cooper *et al.*, 1995b), the quantal output per synapse is greater for the proximal region. The reasons for the higher probability of release per synapse are not fully elucidated, but could include electrical differences, larger inflow of Ca^{2+} at some synapses in the proximal region (Cooper *et al.*, 1995b), and synaptic structural complexity. The last topic will be considered in more detail in the next section.

Aspects of synaptic plasticity

Activity-induced changes in synaptic performance are commonly found in crustaceans, but differ considerably according to the type of motor neuron and specific terminals of an individual neuron (Atwood, 1965; Atwood and Bittner, 1971). The major activity-dependent changes described can be summarized briefly:

(1) Short-term facilitation. Enhancement of release with trains of stimuli or paired pulses. More prominent in tonic than in phasic terminals, and most prominent in the low-output terminals of tonic axons (Atwood and Bittner, 1971). The mechanism of the effect is currently not resolved (Winslow *et al.*, 1994).

(2) Augmentation and potentiation. Different phases of decay of a potentiated EPSP following tetanic stimulation, observed in tonic terminals (Zucker *et al.*, 1991; Delaney and Tank, 1994). Generally attributed to slow removal of Na^+ and Ca^{2+} from the terminal following a bout of activity (Mulkey and Zucker, 1992).

(3) Long-term facilitation, long-term potentiation. A persistent (hours-long) enhancement of transmission induced by tetanic stimulation (Sherman and Atwood, 1971). Apparently not dependent on the build-up of intracellular Ca^{2+} (Wojtowicz and Atwood, 1988), but dependent on adenylyl cyclase activity (Atwood *et al.*, 1989) and possibly involving local synaptic structural modification

(Wojtowicz *et al.*, 1989, 1994).

(4) Low-frequency depression. Decrease in transmitter release following a single impulse, observed for EPSPs of certain phasic terminals. The mechanism is unknown, but probably does not involve depletion of transmitter (Zucker and Bruner, 1977).

(5) High-frequency depression. Progressive decline in EPSP amplitude with maintained stimulation (usually at 5 Hz or higher). Prominent for phasic axons, not for tonic axons. Probably involves limitations in energy supply and hence in maintenance of transmission (Atwood and Nguyen, 1995).

(6) Long-term adaptation. Conversion of synaptic properties in response to altered impulse activity. The best-known examples involve conversion of the terminals of phasic motoneurons to a more tonic phenotype in response to repetitive bouts of induced extra activity (Lnenicka and Atwood, 1985a, b). The conversion is protein synthesis dependent (Nguyen and Atwood, 1990), and will be discussed in more detail in a subsequent section.

In addition to the foregoing types of activity-dependent synaptic modifications, neurohormonal modulation of synaptic transmission and muscle fiber responsiveness by serotonin (Kravitz *et al.*, 1980; Glusman and Kravitz, 1982), octopamine (Breen and Atwood, 1983), proctolin (Bishop *et al.*, 1987) and other agents is also prominent in crustacean neuromuscular systems. The effects of neurohormones and neuromodulators will not be considered further in the present review.

Variation in synaptic structural complexity

To determine structural correlates for the observed physiological differences between phasic and tonic terminals and among terminals of a single motor neuron innervating different targets, we have marked sites of focal recording on the terminal with fluorescent beads to allow us to assess the underlying synaptic structure. These beads can be discerned with fluorescence and electron microscopy (Wojtowicz *et al.*, 1994; Cooper *et al.*, 1995b). It is then possible to serially reconstruct, at the ultrastructural level, the recorded terminal region. An electron micrograph of the phasic and tonic terminals of the leg extensor is shown in Fig. 3. The presynaptic area, synaptic vesicles, mitochondria, and a synaptic dense body (located at an active zone) can easily be discerned. The synaptic active zone is believed to be the site of vesicle release during evoked stimulation because of the clustering of synaptic vesicles and images of vesicular release as well as the localized high density of calcium-activated potassium channels (Robitaille *et al.*, 1993) and putative voltage-gated calcium channels (Robitaille *et al.*, 1990; Cohen *et al.*, 1991; Fariñas *et al.*, 1993; Haydon *et al.*, 1994). To date, the majority of 'direct' structure–function correlations have been obtained at the crayfish opener excitatory motoneuron (Wojtowicz *et al.*, 1994; Cooper *et al.*, 1995b). The most compelling structural correlations with synaptic efficacy are seen in comparing low-output and high-output varicosities: the latter possess more synapses with one or more active zones (i.e., 'complex' synapses). Likewise, there are fewer synapses without any active zones (i.e., 'blank' synapses) in the high-output varicosities (Fig. 4; Table 1). Ultrastructural samples from excitatory terminals have shown that there are more dense bodies per 100 synapses in the terminals on proximal muscle fibers as compared to terminals on central fibers (Table 1). This enhanced synaptic complexity in terminals on proximal fibers is

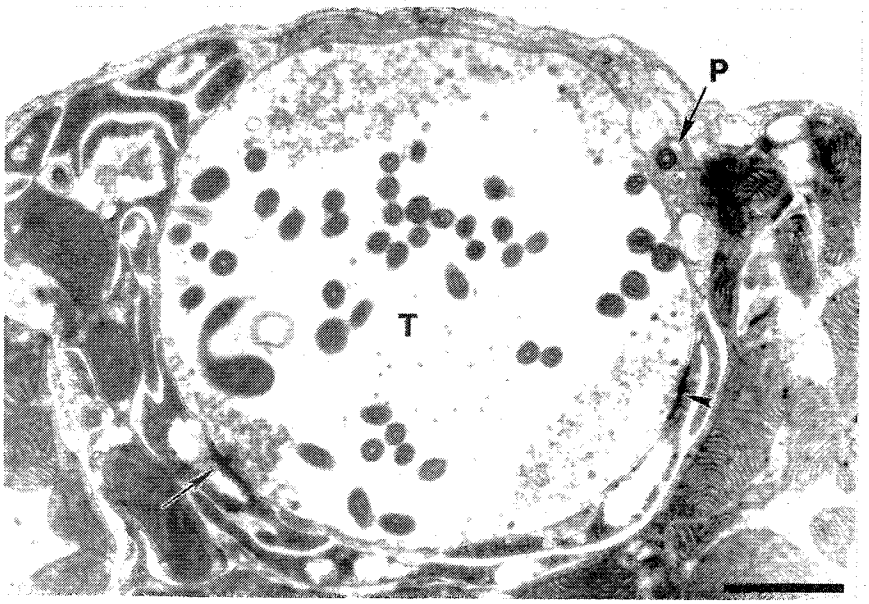

Fig. 3. Electron micrograph of tonic (T) and phasic (P) terminals of the crayfish leg extensor muscle. *The larger tonic terminal has two synapses: one (arrowhead) without an obvious active zone in this section, and the other (arrow) with a presynaptic dense body at a putative active zone. Many mitochondrial profiles appear in the tonic terminal. The small phasic terminal (P) has no synapses at this location, and contains a single mitochondrial profile. Scale bar: 1 μm.*

most likely the reason for the larger Ca^{2+} influxes measured in the terminals at various stimulation frequencies (Cooper *et al.*, 1995b). In addition, the quantal content differences measured along the length of a single terminal located on central muscle fibers (Fig. 5) are associated with a larger number of complex synapses in the varicosities close to the main axon (Cooper *et al.*, 1995b).

Preliminary evidence (Msghina *et al.*, 1995; M. J. King *et al.*, unpublished observations) suggests that the same type of structural differentiation occurs in the phasic and tonic terminals of the leg extensor preparation: the phasic terminals contain a greater proportion of complex synapses.

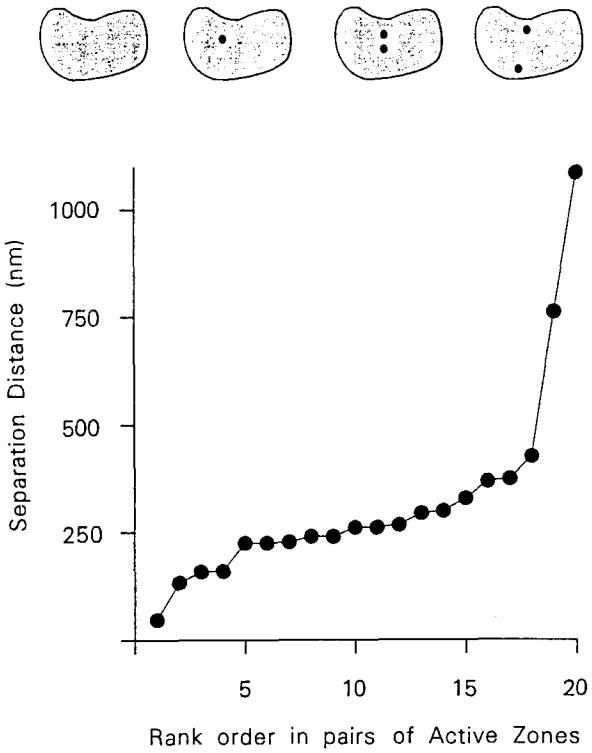

Fig. 4. Variation in the occurrence and placement of presynaptic active zones in crustacean synapses. *Face views of synaptic contact areas (shaded areas) illustrate, from left to right: no active zone, one active zone, two closely spaced active zones, and two separated active zones. The graph plots separation distances of paired active zones for synapses of the crayfish opener muscle in rank order, to illustrate the continuous variation in separation distance for synapses with more than one active zone.*

Measurements made by calcium-sensitive indicators during repetitive stimulation support the notion of functional calcium channels localized at active zones (Llinás *et al.*, 1992; Smith *et al.*, 1993). Since we measured a wide variation (70 to 1000 nm) in the separation distances among active zones of complex synapses (Fig. 4), it is plausible that during electrical activity, closely spaced active zone pairs may have interacting, localized calcium ion concentrations (domains) that result in an increase in the local calcium concentration. This in turn enhances the probability of a vesicle 'detecting' the elevated calcium concentration, an event which can lead to vesicle fusion and subsequent transmission of a quantal unit. Active zones spaced farther apart are more likely to act independently at low frequency stimulation. The hypothesis of active zone interaction was tested with computational analysis for structures and physiological data obtained from the tonic excitatory opener motor nerve terminal in crayfish (Cooper *et al.*, 1996). It was estimated that there was significant interaction among active zones spaced ≤200 nm. Thus, structural complexity of individual synapses may be one determinant of their physiological responsiveness.

Activity-dependent influences on synaptic properties

Short-term facilitation (enhancement of transmitter release following one impulse of a short train) is often attributed to the effects of residual calcium following action potential induced opening of Ca^{2+} channels (Zucker and Lara-Estrella, 1983). Whether the residual-calcium effect is attributable to free Ca^{2+}, or to an effect induced by free Ca^{2+} but outlasting its presence, is currently a hotly debated issue. On the one hand, experiments based upon measurement of calcium-activated potassium conductance ($g_{k(Ca)}$) in the terminal (as a measure of free Ca^{2+} near the terminal membrane) indicate that facilitation of transmitter release outlasts $g_{k(Ca)}$ (Blundon *et al.*, 1993). This implies that free Ca^{2+} disappears very rapidly from submembrane locations, but activates a longer-lasting process which enhances transmitter release. This result is consistent with kinetic studies (Bittner and Sewell, 1976) and effects of Ca^{2+} buffers (Winslow *et al.*, 1994) which also question the need for continued presence of free Ca^{2+} as a necessary permissive agent for facilitation. On the other hand, experiments with photo-activated Ca^{2+} buffers (Kamiya and Zucker, 1994) suggest that sudden removal of free Ca^{2+} from the terminal terminates short-term facilitation, implying that the process requires continued presence of free Ca^{2+}. Resolution of this impasse awaits further experimentation. The end result of facilitation-inducing Ca^{2+} entry is increased transmitter release for up to 1 s after the initial stimulation (in the crayfish opener muscle).

Analyses of quantal release at individual varicosities have suggested an increase in responding synapses

Terminal Morphology	Proximal (high-output)	Central (low-output)
# of synapses/varicosity	29	35
Mean synaptic surface area (μm^2)	0.5	0.3
# of dense bodies/100 synapses	38	16
% of synapses/varicosity with and without active zones		
# of AZ's		
0	10%	31%
1	38%	51%
2	38%	14%
3	14%	3%

Table 1. Structural differences of high- and low-output varicosities (modified after Cooper *et al.*, 1995b).

during short-term facilitation. A binomial model of release (Cooper *et al.*, 1995c) provides the best description of the variable release observed at crayfish terminals (Wernig, 1975; Smith *et al.*, 1991; Atwood *et al.*, 1994). In this model, quantal content (m) is the product of n (units available for release) and p (mean probability of release). Recently, n has been taken to represent the number of respondent active zones. At low frequencies of stimulation, n is small for crayfish opener axon varicosities (usually less than 5; Table 2). The values for n are much smaller than those for the number of synapses with active zones on a varicosity.

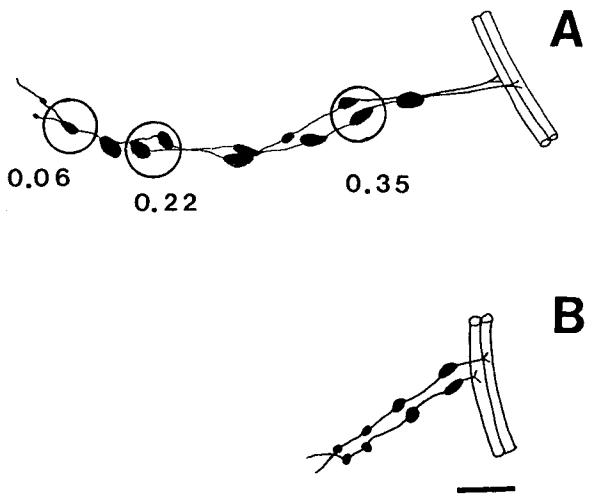

Fig. 5. Strings of varicosities in the opener muscles of intermediate-sized (A) and small (B) crayfish, drawn from 4-Di-2-Asp stains of the central region of the muscle. *Intermediate-sized (5 to 6.5 cm) and small-sized (3.5 to 4 cm) crayfish are measured from rostrum to telson, inclusive. Paired strings arise from the excitatory and inhibitory axons, with approximately equal numbers of excitatory and inhibitory varicosities. Recordings along the string in A with a macro-patch electrode (placements indicated by circles) illustrate non-uniformity of quantal release at 1 Hz (quantal content values are indicated). The distal varicosities typically have a much lower quantal content than varicosities near the point of origin of the terminal.*

With increased frequency, short-term facilitation enhances the release of transmitter (frequency facilitation). The binomial analyses show higher values for both n and p (Wernig, 1972; Hatt and Smith, 1976; Wojtowicz *et al.*, 1994; Atwood *et al.*, 1994); an example is provided in Table 2.

The increase in p can reasonably be associated with persistent Ca^{2+}, which has been demonstrated by calcium imaging to remain in the terminal after stimulation (Delaney *et al.*, 1989; Cooper *et al.*, 1995b), though not necessarily at a high enough concentration to activate release at the presynaptic membrane.

An hypothesis for the increase in n can be drawn from the structural data (Fig. 4, Table 1). Synapses of a varicosity include some with two or more active zones (for central synapses in Table 1, 17%). The spacing of active zones in these 'complex' synapses is variable (Fig. 4). As noted in the previous section, interaction of local Ca^{2+} domains is more likely for closely spaced active zones, and synapses possessing them would thus be more likely to release transmitter. As stimulation proceeds, and the low-level background of Ca^{2+} increases, more distant pairs of active zones would be more likely to interact. At even higher frequencies, or with more prolonged stimulation, synapses with a single active zone would be more likely to participate in release. Thus, short-term facilitation may involve progressive recruitment of synapses. The large number of synapses on crayfish terminals provides broad scope for such a mechanism; and in fact, the amount of facilitation obtained in crustacean 'tonic' terminals exceeds that of most other neurons studied to date.

A contrasting situation occurs with long-term facilitation (or long-term potentiation). Inducing stimulation typically leads to a 50–100% enhancement of release (Table 2) which persists for at least several hours (Atwood and Wojtowicz, 1986). The binomial analyses have consistently shown an increase in n, with little or no change in p (Wojtowicz and Atwood,

Short-term frequency facilitation:

Stimulation rate	Mean quantal content	Estimated number of release sites	Estimated mean probability of release
Hz	*m*	*n*	*p*
2	0.051	1	0.05
4	0.150	2	0.075
10	0.56	6	0.09
20	1.33	10	0.13

Long-term facilitation:

Stimulation rate	Mean quantal content	Estimated number of release sites	Estimated mean probability of release
Hz	*m*	*n*	*p*
Control (5 Hz test)	1.82	4	0.45
After LTF (5 Hz test)	2.81	6	0.47

Table 2. Quantal parameters effected by short-term and long-term facilitation (modified after Wojtowicz *et al.*, 1994).
To induce LTF the nerve was stimulated at 20 Hz for 10 min.

1986; Wojtowicz *et al.*, 1988). How this comes about is not fully known, but a structural correlate appears to be a larger proportion of 'complex' synapses (Wojtowicz *et al.*, 1994). Addition of active zones may involve splitting of some already present. Changes in phosphorylation of synaptically important molecules very likely occurs (Atwood *et al.*, 1989), but the targets for phosphorylation are not presently known.

The overall contrast between short-term facilitation and long-term facilitation (long-term potentiation) is that synapses are transiently recruited to service in the former case, in conjunction with an over-all increase in release probability (occasioned by build-up of the Ca^{2+} background in the terminal). In contrast, during the long-term effect a semi-permanent change in the number of easily recruitable synapses appears to be important (increase in *n* without a change in mean *p*).

Long-term adaptation of phasic motor neurons

Heightened synaptic input to a postsynaptic neuron can result in long-lasting structural changes as was shown for Purkinje cells in the cerebellum (Sotelo, 1978) and hippocampus (Geinisman *et al.*, 1992). When the postsynaptic cell is a muscle fiber, striking biochemical and structural changes occur with alteration in electrical activity; this is particularly well documented in mammalian muscles (Burke, 1981). In crustacean motor systems, alteration of muscle fiber properties with activity has not been studied in adult animals, though it appears to be important during early development (Ogonowski *et al.*, 1980; Govind, 1982). However, the presynaptic motor nerve terminals that are responsible for conducting electrical activity and synaptic transmission show structural and physiological alterations when electrical activity is altered; this has been investigated in crayfish phasic motor neurons (Lnenicka and Atwood, 1985b; Nguyen and Atwood, 1990; Goelet *et al.*, 1986).

Long-term adaptation (LTA) comprises a series of activity-induced changes that persist for days or weeks in presynaptic neurons; particularly striking changes occur at phasic neuromuscular junctions in crayfish after periodic or sustained increases in electrical activity (Lnenicka and Atwood, 1985b). The converted phasic neuron produces an initial EPSP of reduced amplitude, and the response to repetitive stimulation shows fatigue resistance as well as increased facilitation (Lnenicka and Atwood, 1985a, b; Mercier and Atwood, 1989). These two physiological changes appear with different time courses, and thus probably represent two distinct processes (Nguyen and Atwood, 1992b). Both changes shift the physiology of the phasic terminals towards a more tonic phenotype. In addition, morphological transformation gives the phasic terminals a more tonic-like appearance. The transformation of the terminal's morphology from thin filiform to varicose is accompanied by an increase in mitochondrial cross sectional area and branching (Lnenicka *et al.*, 1986; Atwood *et al.*, 1991). The persistent physiological changes were shown to be independent of synaptic transmission (Lnenicka and Atwood, 1988) but dependent on protein synthesis

(Nguyen and Atwood, 1990; see Fig. 6). Blocking protein synthesis prior to the onset of conditioning stimulation retards the alteration whereas a later block is ineffective, indicating that a critical period is present. Consequently, there is a strong indication that gene regulation is involved, as in other neural systems showing long-lasting activity-related changes (Goelet *et al.*, 1986). The overall steps in the transformation are outlined in Fig. 6.

The search for underlying mechanisms for these alterations entails attempting to define changes in synaptically significant proteins and changes in the regulation of the genes responsible for producing them.

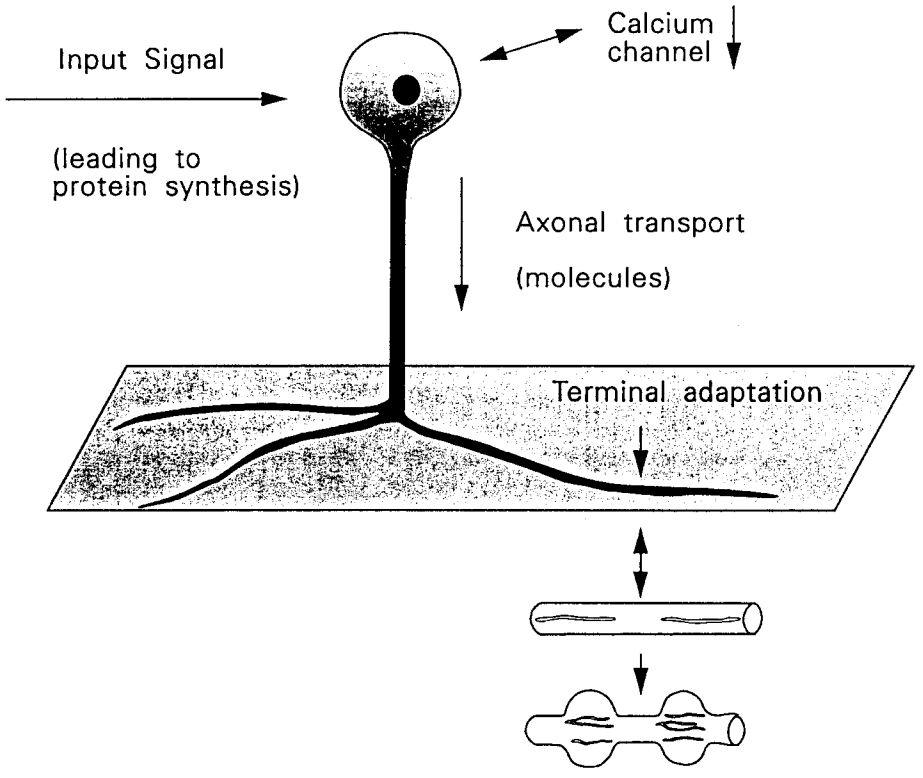

Fig. 6. Long-term adaptation of crayfish phasic motor neurons. *Activity-dependent changes impinging on the central part of the neuron lead to changes in protein synthesis, transport of molecules to the terminals, and alterations in the terminals, including enlargement of the mitochondria and terminal varicosities.*

There are a number of proteins which could in principle be responsible for the conversion in physiology and morphology of the phasic motor neurons. Both the initial reduction in EPSP amplitude and the increase in fatigue resistance could be linked to reduction in the number of active calcium channels in the presynaptic terminals. Studies in the moth, *Manduca*, and in the lizard, *Anolis*, have shown that the number of intermembranous particles (putative calcium channels) at active zones (Pumplin *et al.*, 1981) is smaller in tonic than in phasic neuromuscular junctions (Rheuben, 1985; Walrond and Reese, 1985). Other features which could be relevant, if altered, include calcium buffering and calcium extrusion (Atwood and Lnenicka, 1992). Differences in phosphatases that can dephosphorylate synapsins could also lead to altered EPSP amplitude (Llinas *et al.*, 1985; Hackett *et al.*, 1990; Swain *et al.*, 1991). Proteins associated with mitochondria may show differences in expression. That mitochondria are likely to be involved in the transformation is indicated by an increase in their size and branching, and by their increased ability to take up fluorescent dyes such as rhodamine (Nguyen and Atwood, 1992a). Mitochondrial changes could account for the increase in the fatigue resistance of the transformed phasic terminals. In order for the long term adaptations to take place in the crayfish phasic motor neurons, Ca^{2+} influx is necessary in the soma (Hong and Lnenicka, 1993). It has been demonstrated that increased electrical activity resulted in Ca^{2+} currents in the soma being down-regulated, an effect which is protein synthesis-dependent (Nguyen and Atwood, 1990). It is not yet known whether such an effect occurs also at the peripheral synapses of the same neuron. Such an effect could provide negative feedback over longer periods of conditioning. The results imply that one or more proteins are up- or down-regulated to reduce the Ca^{2+} currents in response to the heightened electrical activity (Hong and Lnenicka, 1995).

To determine which proteins are actually involved in the transformation, one can employ a number of experimental approaches. One approach is to compare and isolate [^{35}S]-methionine labeled proteins on 2-D electrophoretic gels. This has been used successfully for groups of cells in the *Aplysia* nervous system (Castellucci *et al.*, 1988). Changes in protein synthesis

have been shown to occur in *Aplysia* neurons during conditioning (Castellucci *et al.*, 1988). Induction of long-term facilitation of the appropriate *Aplysia* CNS neurons *in vitro* resulted in the regulation of expression of at least 15 proteins (Barzilai *et al.*, 1989). At least one of these was found to be similar to a mammalian neural adhesion molecule (NCAM), and its down-regulation was linked to morphological changes (more extensive branching) in the responding nerve cells (Doherty *et al.*, 1995). The advantage of this approach is that if proteins can be identified, the final outcome of differential gene regulation can be defined in terms of expressed proteins, which can be related to cellular functions in many cases, as in the example from *Aplysia*. The disadvantages are that the approach is time-consuming and expensive, requires repeated replications to assess reliability (Castellucci *et al.*, 1988), and requires relatively large amounts of the proteins for detection, due to low resolution. In addition, once the size and charge of the proteins have been determined from the 2-D gel, the task of protein identification through sequencing or other means must be undertaken.

Other approaches to address altered expression of significant proteins have been based upon changes in the expression levels of mRNAs which code for specific proteins. Some commonly used approaches are 'differential screening' and 'subtractive hybridization' (Sambrook *et al.*, 1989). These two approaches have a potential disadvantage: if a single mRNA is abundantly expressed, many of the clones that are sequenced will contain the same DNA sequence, and rare clones may be missed. Analyses at the single-cell level would be affected by this limitation. A way to avoid losing rare mRNAs would be to use subtracted libraries in which rare mRNAs are enriched (Sambrook *et al.*, 1989). This approach has been used to examine differences among identified neurons in the leech CNS (Korneev *et al.*, 1994).

A relatively new approach which offers a way around some of the above limitations is to amplify all the intracellular mRNAs approximately a million-fold and to screen for several known sequences of particular interest simultaneously (Eberwine *et al.*, 1992), using available cDNA probes for molecules of interest. This technique has proven to be successful in examining mRNA levels in Purkinje neurons (Van Gelder *et al.*, 1990), and in cells of the hippocampus (Mackler *et al.*, 1992; Eberwine *et al.*, 1992). Variations in this approach are also being used (Monyer and Lambolez, 1995; Sucher and Deitcher, 1995). A preliminary report indicated that this approach, which can be referred to as 'expression profiling by dot-blot hybridization', is feasible for lobster neurons (Kravitz *et al.*, 1992). A disadvantage for the crayfish system is that, for optimal results, one needs known cDNA sequences from this species for the dot-blot hybridizations. One cannot assume sufficiently high homology between crayfish cDNAs and those of other distant species.

Some attempts have been made to test protein similarity in *Drosophila* (where many proteins and their genes are known) and crayfish (where few are presently known). An example is the synaptic protein synaptotagmin. Antibodies raised against synaptotagmin from *Drosophila* showed selective staining in regions of the crayfish motor nerve terminals where ultrastructural work has shown that synaptic vesicles are probably located, indicating the possibility of a close homology of the epitope regions of this molecule for these two species (Fig. 7). However, the *Drosophila* and crayfish proteins did not show the same molecular weight on Western blots, so they are different despite the fact that they both bind the *Drosophila* antibody (Cooper *et al.*, 1995a). This example suggests that even conserved synaptic proteins can be expected to show differences, though homology between crustacean and *Drosophila* proteins is relatively high in

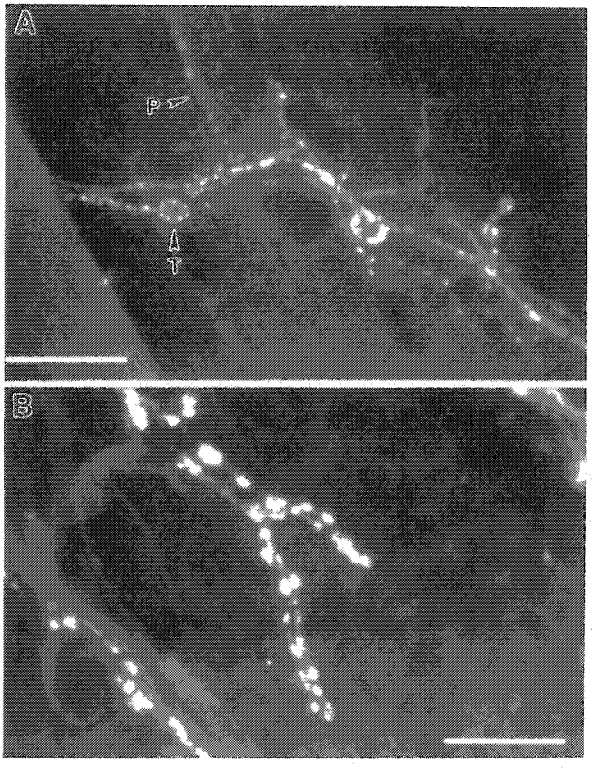

Fig. 7. Synaptotagmin (*Drosophila*) antibodies stain phasic (P) and tonic (T) terminals of the crayfish leg extensor (A), and excitatory and inhibitory terminals of the crayfish opener muscle (B). *Within the large tonic varicosities, localized regions of high-intensity staining are visible. These probably represent locations of one or more synapses. Scale bars: 25 μm for A and B.*

other reported cases (Pelliccia *et al.*, 1991; Hariyama *et al.*, 1993; Kang and Naya, 1993; Okimoto and Wolstenholme, 1993; Helfrich-Forster and Homberg, 1993; Cotton and Mykles, 1993; Manzanares *et al.*, 1993; Van Raay and Crease, 1994; Ramon Valverde *et al.*, 1994; Finbow *et al.*, 1994; Luck *et al.*, 1995).

A recent approach known as 'differential display' (Liang and Pardee, 1992; Liang *et al.*, 1993) has proved to be of particular interest in examining expression differences among cells with altered states. In brief, this method uses reverse transcription of an anchored oligo-dT primer with one or two 3′ bases to recognize a fraction of the 3′ poly (A) sequence of mRNAs. This fraction can be further subdivided and amplified by use of a decamer oligodeoxynucleotide of arbitrarily defined sequence which anneals to the 5′ end of the amplified fragments. These subfractions can then be separated by size on a denaturing polyacrylamide gel. Through use of multiple combinations of primer sets, one is potentially able to visualize all the expressed mRNAs within the starting material. This approach permits detection of up- as well as down-regulated expression of mRNAs through comparison of the banding patterns of control and experimental tissues. The disadvantage of this approach is that primarily the 3′ end of the mRNA is amplified, and this may not contain the coding region for the protein. In addition, one does not know what the potentially numerous multiple bands code for until full sequencing is obtained for each band.

In addressing the issue of which proteins are up- and down-regulated during the transformation of the phasic motor neuron in the crayfish, we have used both the expression profiling and the differential display techniques at the single cell level (Pekhletsky *et al.*, 1994; Pekhletski *et al.*, 1995). The soma of a single large identified phasic motor neuron located within each of the abdominal segmental ganglia, the 'common excitor' of the abdominal deep extensor muscles (Parnas and Atwood, 1966), was used. The terminals of this neuron, like those of other identified abdominal phasic neurons (Mercier and Atwood, 1989) are thought to undergo characteristic long-term adaptation with conditioning stimulation. The use of a single large identified neuron (80–100 μm in diameter) permits the paired neuron in the other half of the ganglion to be used as a symmetrical unconditioned control. This is an additional advantage of the crayfish for single-cell studies.

Initially, the expression profiling approach was checked to determine whether the hybridizations are meaningful when known cDNAs for vertebrate species and *Drosophila* are used. With enough starting material, Northern blotting could have been used, but since this was not the case at the level of single cells, the amplified unknown crayfish cDNAs which hybridized to the probes were extracted and sequenced. The sequencing data revealed that most of the signals were false positives even under highly stringent hybridization conditions (Pekhletsky *et al.*, 1994). More recently, we employed the differential display approach to compare single identified transformed phasic motor neurons and their contralateral controls. Preliminary evidence shows an up-regulation of several mRNAs and a down-regulation of others (Pekhletski *et al.*, 1995). The full coding sequences of interest in the multiple bands have yet to be fully sequenced, but it is likely that several proteins are affected in their expression by conditioning stimulation.

The hypothesis that we are testing is that increased neuronal activity, which produces the phasic to tonic motor neuron transformation, results in altered expression of mRNAs. Those mRNAs that are regulated must code for pertinent proteins that allow the interconversion to take place. Some possible proteins which may become altered are: voltage sensitive Ca^{2+}, Na^{+} and K^{+} channels; vesicle associated proteins (i.e., VAMPS, syntaxins, synaptotagmin, and NSF); mitochondrial associated proteins including cytochrome oxidase proteins; and proteins known to act as second messengers including calcium calmodulin-dependent kinases.

An understanding of the cellular mechanisms regulating transcription of mRNAs that result in the translation of proteins, in a well defined *in vivo* system with identifiable motor neurons, would provide an insight into the molecular differences between types of motor neurons with different activity patterns and the changes that occur during activity-dependent transformation. Crayfish motor neurons provide an advantageous system from the standpoint of single-cell analysis, since much is known of their morphology and physiology. Application of molecular biological approaches will, in due course, provide additional insights into the factors responsible for differential synaptic performance.

Developmental and trophic influences on synaptic properties

Crayfish neuromuscular junctions can also be used to investigate synaptic performance and structural differences during development, maturation, and regeneration (Atwood and Kwan, 1976; Stewart and Atwood, 1992; Cooper *et al.*, 1995b). For example, on the central fibers of the opener muscle the strings of varicose terminals are shorter in young animals than in older

ones (Fig. 5). In the proximal muscle fibers there also appears to be an elongation of the varicose terminals with age. This prominent increase in growth of motor nerve terminals with age is also observed in other crustaceans, especially the American lobster (Govind and Walrond, 1989; Govind, 1992).

In view of the substantial terminal structural differences in a single neuron contacting different targets, as in the example of the proximal and central crayfish opener muscle fibers, one can speculate that there is selectivity in axonal transport to the two regional terminals and/or that there is a differential feed-back from the regionally distinct muscle fibers. The hypothesis of target influences on transmission properties of nerve terminals was introduced for crustacean muscles by Frank (1973). This possibility is supported in crayfish by target differences; the proximal and central muscle fibers of the opener muscle are found to be histochemically different in their fiber type composition in another crayfish species (Günzel *et al.*, 1993). Intrinsic differences in target cells could then promote local regulation of the nerve terminals. Such a mechanism is also implied for two terminals of a single motor neuron in the crustacean stomatogastric system: one terminal branch innervating a defined target muscle displays facilitation, while another branch to a separate target muscle shows depression (Katz *et al.*, 1993). This is also the case for a sensory neuron innervating different target neurons within the CNS of crickets (Davis and Murphey, 1993). In the preparations with dual phasic and tonic innervation of the same muscle fibers, the case for retrograde feedback from target cells is less easy to envision. Instead, it is more likely that intra-neuronal differences are responsible for the resulting morphology and physiology. For phasic and tonic motor nerve terminals, intrinsic neuronal differences are probably playing the major role in for determining the differences in varicosity size and synaptic output. The topic of retrograde signalling has been reviewed recently by Cooper *et al.* (1992), Connor and Smith (1994), Davis and Murphy (1994), and Grinnell (1995).

The developmental constraints of the motor nerve terminals have substantial effect on the general terminal morphology and underlying synaptic ultrastructure which influences the efficacy of transmission. A good example to illustrate the relationship of varicosity age and synaptic efficacy is summarized in Fig. 5. The terminals on the central opener muscle fibers display a gradation in mean quantal output along the length of the terminal. The most distal varicosities on the terminal have the lowest quantal content and are the most recently formed developmentally. Serial reconstructions of a low-output varicosity contained fewer complex synapses (Table 1) as compared to older, high-output varicosities (Cooper *et al.*, 1995b). This phenomenon also occurs in regenerating limbs of the shore crab, *Grapsus*, where a distinct temporal pattern of synaptic differentiation with maturation was observed (Govind *et al.*, 1973). The developing terminals displayed low synaptic output, but as the limb developed, synaptic transmission became enhanced, with fewer failures during stimulation.

In trying to decide how the morphological differences arise among the terminals on proximal and central opener muscle fibers, one is left believing that there must be a continuous bidirectional exchange of information between the nerve and muscle. One may imagine that the muscle can 'sense' when it is satisfied with the amount of stimulation and in response, limit the growth of the nerve terminals. The stimulation efficacy may in some way be measured by changes in the muscle fiber membrane potential. The terminals on the proximal fibers, which are more cluster-like in appearance and possess fewer varicosities per muscle fiber, may be inhibited from forming long strings by a feed-back signal linked to membrane depolarization. The larger depolarization of proximal fibers is due in part to their higher input resistance; they produce a larger postsynaptic response for a given current than do central fibers.

Developmental influences are also likely to play a role in terminal formation. For example, in the crayfish leg extensor muscle, the tonic terminal is parallel with the phasic, but when the tonic terminal ends, the phasic terminal continues beyond the last tonic varicosities. In fact, there is substantially more terminal length of the phasic than of the tonic terminals in the leg extensor preparations. In addition, one often sees the varicose tonic terminal encircled by the thin phasic terminal, which is a strong indication that the phasic terminals follow guidance cues of the tonic terminal during development. This pattern of innervation is not unique to crustaceans, since it is also seen in the innervation of larval *Drosophila* muscles 6 and 7 (Kurdyak, 1993; Fig. 4). Muscles 6 and 7 are innervated by two distinct excitatory motor axons which produce two types of nerve terminals (Ib and Is). The Ib (big varicosity) terminals are analogous to tonic terminals of the crayfish both physiologically and morphologically, whereas the Is (small varicosity) type is physiologically more like the crustacean phasic terminals, with higher output of transmitter and relatively smaller varicosities (Lnenicka *et al.*, 1986; Atwood *et al.*, 1993; Kurdyak *et al.*, 1994). In this preparation, the Is terminal is seen occasionally encircling the Ib, implying that it comes later in development. Thus, studies from *Drosophila* (Halpern *et al.*, 1991;

Keshishian *et al.*, 1993) suggest parallels with crustaceans in synapse formation.

In the leg extensor, besides the tonic and phasic excitatory innervation, there is inhibitory innervation as well. On some single muscle fibers there are three terminals, side by side in places, with different synaptic physiological properties. Preliminary work (Bradacs *et al.*, 1995; M. Msghina and H. L. Atwood, unpublished observations), using immunocytochemistry, has shown that the inhibitory motor nerve terminals are varicose in nature in the leg extensor. The varicose nature of inhibitory terminals is commonly observed in other specific inhibitory motor neurons such as those in the leg opener (Cooper *et al.*, 1995a, b) and the distal accessory flexor muscle in lobster (Walrond *et al.*, 1993). The varicose formation appears to be intrinsic to tonic nerve terminals and can be induced to occur in phasic neurons that are exposed to tonic-like activity, as explained in the previous section. Therefore, it appears that activity patterns of the neuron (a presynaptic factor) can help to determine the morphological and physiological characteristics of the terminal.

In summary, the developmental stage, along with target influences and intrinsic neuronal differentiation, undoubtably all have significant effects in determining the physiological properties of motor nerve terminals.

Conclusions

In this review, we have summarized viewpoints about synaptic performance and its alteration which have developed from observations on crustacean motor neurons. For investigations of synaptic transmission and plasticity, crustacean neurons continue to be excellent models.

A major theme presented here is that individual synapses can be recruited (and decommissioned) in response to functional demands. The nervous system has a great many more individual synapses at its disposal than are used for normal activity. Results from the crustacean neurons suggest that many of them are effectively 'silent' during normal activity. With increased activity, more are recruited (short-term facilitation). A working hypothesis is that individual synapses are not all equally likely to respond to a single nerve impulse by releasing one or more quantal units of transmitter. Some synapses on each nerve terminal have more complex structure than others. We hypothesize that the more complex synapses (particularly those with two or more closely spaced active zones) are more likely to respond than less complex synapses. A plausible explanation derives from the overlapping calcium domains of closely adjacent active zones.

This hypothesis leads to a possible explanation for the difference in quantal emission of 'high-output' and 'low-output' varicosities of the same tonic motor neuron. Structural analysis indicates a higher proportion of complex synapses on 'high-output' varicosities. There are no other obvious structural differences. However, when the comparison is extended to phasic motor neurons, a more extreme situation is evident; for an equivalent number of individual synapses, quantal release at low frequencies can be 50–100 times higher. The hypothesis based upon structural complexity is probably inadequate to account for such a large difference in quantal emission, and it is likely that molecular differences between phasic and tonic neurons play an important role in establishing the probability of synaptic release.

Maintained stimulation leads to synaptic depression in phasic axons, and to potentiation and long-term facilitation (long-term potentiation) in tonic axons. Long-lasting enhancement may be accompanied by increased structural complexity in a few synapses. Depression in phasic terminals appears to be linked to mitochondrial function, which is less robust in these neurons.

Neuronal adaptation to persistently increased impulse activity is strikingly evident in phasic neurons, which acquire more of the characteristics of tonic neurons. Increased fatigue resistance probably derives from alterations in mitochondrial properties and their increased volume in the terminals. Other changes are currently less well defined. We have outlined a strategy for defining molecular changes in single identified neurons.

The establishment of synapses with specific physiological characteristics on defined target cells is a striking feature of crustacean neuromuscular systems. We have presented evidence for involvement of both presynaptic influences and postsynaptic influences, but further definitive experimental and developmental work is needed to answer current questions on this topic.

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References

Atwood, H. L. (1965) Excitation and inhibition in crab muscle fibres. *Comp. Biochem. Physiol.*, **16**, 409-426.

Atwood, H. L. (1967) Variation in physiological properties of crustacean motor synapses. *Nature*, **215**, 57-58.

Atwood, H. L. (1972) Crustacean muscle. In *The Structure and Function of Muscle*, ed. G. H. Bourne, pp. 421-489. New York: Academic Press.

Atwood, H. L. (1973a) Crustacean motor units. In *Control of Posture and Locomotion*, ed. R. B. Stein, K. G. Pearson, R. S. Smith and J. B. Redford, pp. 87-104. New York: Plenum Press.

Atwood, H. L. (1973b) An attempt to account for the diversity of crustacean muscles. *Amer. Zool.*, **13**, 357-378.

Atwood, H. L. (1976) Organization and synaptic physiology of crustacean neuromuscular systems. *Prog. Neurobiol.*, **7**, 291-391.

Atwood, H. L. and Bittner, G. D. (1971) Matching of excitatory and inhibitory inputs to crustacean muscle fibers. *J. Neurophysiol.*, **34**, 157-170.

Atwood, H. L. and Hoyle, G. (1965) A further study of the paradox phenomenon of crustacean muscle. *J. Physiol. (Lond.)*, **181**, 225-234.

Atwood, H. L. and Kwan, I. (1976) Synaptic development in the crayfish opener muscle. *J. Neurobiol.*, **7**, 289-312.

Atwood, H. L. and Lnenicka, G. (1992) Differential effects of calcium entry on phasic and tonic motor axons of crayfish. *Soc. Neurosci. Abstr.*, **18**, 246.

Atwood, H. L. and Nguyen, P. V. (1995) Neural adaptation in crayfish. *Amer. Zool.*, **35**, 28-36.

Atwood, H. L. and Walcott, B. (1965) Recording of electrical activity and movement from legs of walking crabs. *Can. J. Zool.*, **43**, 657-665.

Atwood, H. L. and Wojtowicz, J. M. (1986) Short-term and long-term plasticity and physiological differentiation of crustacean motor synapses. *Int. Rev. Neurobiol.*, **28**, 275-362.

Atwood, H. L., Dixon, D. and Wojtowicz, J.M. (1989) Rapid introduction of long-lasting synaptic changes at crustacean neuromuscular junctions. *J. Neurobiol.*, **20**, 373-385.

Atwood, H. L., Nguyen, P. V. and Mercier, A. J. (1991) Activity-dependent adaptation in neuromuscular systems: comparative observations. In *Plasticity of Motoneuronal Connections*, ed. A. Wernig, pp. 101-114. Amsterdam: Elsevier.

Atwood, H. L., Govind, C. K. and Wu, C.-F. (1993) Differential ultrastructure of synaptic terminals on ventral longitudinal abdominal muscles in *Drosophila* larvae. *J. Neurobiol.*, **24**, 1008-1024.

Atwood, H. L., Cooper, R. L. and Wojtowicz, J. M. (1994) Non-uniformity and plasticity of quantal release at crustacean motor nerve terminals. In *Molecular and Cellular Mechanisms of Neurotransmitter Release*. ed. L. Stjärne, P. Greengard, S. Grillner, T. Hökfelt and D. Ottoson, pp. 363-382. New York: Raven Press.

Atwood, H. L., Msghina, M., Lindemeier, H. and Pongs, O. (1995) Homologue of *Drosophila* neural protein frequenin selectively expressed in crustacean phasic motor terminals. *Soc. Neurosci. Abstr.*, **21**, 709.6.

Barzilai, A., Kennedy, T. E., Sweatt, J. D. and Kandel, E. R. (1989) 5-HT modulates protein synthesis and the expression of specific proteins during long-term facilitation in *Aplysia* sensory neurons. *Neuron*, **2**, 1577-1586.

Bishop, C. A., Wine, J. J., Nagy, F. and O'Shea, M. R. (1987) Physiological consequences of a peptide cotransmitter in a crayfish nerve–muscle preparation. *J. Neurosci.*, **7**, 1769-1779.

Bittner, G. D. (1968) Differentiation of nerve terminals in the crayfish opener muscle and its functional significance. *J. Gen. Physiol.*, **51**, 731-758.

Bittner, G. D. and Sewell, V. L. (1976) Facilitation at crayfish neuromuscular junctions. *J. Comp. Physiol.*, **109**, 287-308.

Blundon, J. A., Wright, S. N., Brodwick, M. S. and Bittner, G. D. (1993) Residual free calcium is not responsible for facilitation of neurotransmitter release. *Proc. Natl. Acad. Sci. USA*, **90**, 9388-9392.

Bradacs, H., Cooper, R. L., Msghina, M. and Atwood, H. L. (1995) Divergent synaptic transmission and fine structure of phasic and tonic crustacean motor nerve endings to the same postsynaptic target. *Soc. Neurosci. Abstr.*, **21**, 138.14.

Breen, C. and Atwood, H. L. (1983) Octopamine – a neurohormone with pre-synaptic activity-dependent effects at crayfish neuromuscular junctions. *Nature*, **303**, 716-718.

Burke, R. (1981) Motor units: Anatomy, physiology, and functional organization. In *Handbook of Physiology: The Nervous System. Motor Control. Sect. 1., Vol 2*, pp. 345-422. Bethesda: American Physiological Society.

Castellucci, V. F., Kennedy, T. E., Kandel, E. R. and Goelet, P. (1988) A quantitative analysis of 2-D gels identifies proteins in which labeling is increased following long-term sensitization in *Aplysia*. *Neuron*, **1**, 321-328.

Cohen, M. W., Jones, O. T. and Angelides, K. J. (1991) Distribution of Ca^{2+} channels on frog motor nerve terminals revealed by fluorescent ω-conotoxin. *J. Neurosci.*, **11**, 1032-1039.

Connor, E. A. and Smith, M. A. (1994) Retrograde signaling in the formation and maintenance of the neuromuscular junction. *J. Neurobiol.*, **25**, 722-739.

Cooper, R. L., Fernández-de-Miguel, F., Adams, W. B. and Nicholls, J. G. (1992) Anterograde and retrograde effects of synapse formation on calcium currents and neurite outgrowth in cultured leech neurons. *Proc. R. Soc. Lond. B Biol Sci.*, **249**, 217-222.

Cooper, R. L., Hampson, D. R. and Atwood, H. L. (1995a) Synaptotagmin-like expression in the motor nerve terminals of crayfish. *Brain Res.*, **703**, 214-216.

Cooper, R. L., Marin, L. and Atwood, H. L. (1995b) Synaptic differentiation of a single motor neuron: Conjoint definition of transmitter release, presynaptic calcium signals, and ultrastructure. *J. Neurosci.*, **15**, 4209-4222.

Cooper, R. L., Stewart, B. A., Wojtowicz, J. M., Wang, S. and Atwood, H. L. (1995c) Quantal measurement and analysis methods compared for crayfish and *Drosophila* neuromuscular junctions, and rat hippocampus. *J. Neurosci. Methods*, **61**, 66-79.

Cooper, R. L., Winslow, J. L., Govind, C. K. and Atwood, H. L. (1996) Synaptic structural complexity as a factor enhancing probability of calcium-mediated transmitter release. *J. Neurophysiol.*, in press.

Cotton, J. L. S. and Mykles, D. L. (1993) Cloning of a crustacean myosin heavy chain isoform: Exclusive expression in fast muscle. *J. Exp. Zool.*, 267, 578-586.

Davis, G. W. and Murphey, R. K. (1993) A role for postsynaptic neurons in determining presynaptic release properties in the cricket CNS: Evidence for retrograde control of facilitation. *J. Neurosci.*, 13, 3827-3838.

Davis, G. W. and Murphey, R. K. (1994) Retrograde signaling and the development of transmitter release properties in the invertebrate nervous system. *J. Neurobiol.*, 25, 740-756.

Delaney, K. R. and Tank, D. W. (1994) A quantitative measurement of the dependence of short-term synaptic enhancement on presynaptic residual calcium. *J. Neurosci.*, **14**, 5885-5902.

Delaney, K. R., Zucker, R. S. and Tank, D. W. (1989) Calcium in motor nerve terminals associated with posttetanic potentiation. *J. Neurosci.*, **9**, 3558-3567.

Doherty, P., Fazeli, M. S. and Walsh, F. S. (1995) The neural cell adhesion molecule and synaptic plasticity. *J. Neurobiol.*, **26**, 437-446.

Dudel, J. (1982) Transmitter release by graded local depolarization of presynaptic nerve terminals at the crayfish neuromuscular junction. *Neurosci. Lett.*, **32**, 181-186.

Dudel, J., Parnas, I., Cohen, I. and Franke, C. (1984) Excitability and depolarization–release characteristics of excitatory nerve terminals in a tail muscle of spiny lobster. *Pflügers Arch.*, **401**, 293-296.

Eberwine, J., Yeh, H., Miyashiro, K., Cao, Y., Nair, S., Finnell, R., Zettel, M. and Coleman, P. (1992) Analysis of gene expression in single live neurons. *Proc. Natl. Acad. Sci. USA*, **89**, 3010-3014.

Fariñas, I., Egea, G., Blasi, J., Cases, C. and Marshal, J. (1993) Calcium channel antagonist omega-conotoxin binds to intramemebrane particles of isolated nerve terminals. *Neuroscience*, **54**, 745-752.

Finbow, M. E., Goodwin, S. F., Meagher, L., Lane, N. J., Keen, J., Findlay, J. B. and Kaiser, K. (1994) Evidence that the 16 kDa proteolipid (subunit c) of the vacuolar H(+)-ATPase and ductin from gap junctions are the same polypeptide in *Drosophila* and *Manduca*: molecular cloning of the Vha16K gene from *Drosophila*. J. Cell Sci., 107, 1817-1824.

Frank, E. (1973) Matching of facilitation at the neuromuscular junction of the lobster: a possible case for influence of muscle on nerve. *J. Physiol. (Lond.)*, **233**, 635-658.

Geinisman, Y., Morell, F. and DeToledo-Morell, L. (1992) Increase in the number of axospinous synapses with segmented postsynaptic densities following hippocampal kindling. *Brain Res.*, **569**, 341-347.

Glusman, S. and Kravitz, E. A. (1982) The action of serotonin on excitatory nerve terminals in lobster nerve–muscle preparations. *J. Physiol. (Lond.)*, **325**, 223-241.

Goelet, P., Castellucci, V. F., Schacher, S. and Kandel, E. R. (1986) The long and short of long-term memory – a molecular framework. *Nature*, **322**, 419-422.

Govind, C. K. (1982) Development of nerve, muscle and synapse. In *The Biology of Crustacea, Vol. 3, Neurobiology: Structure and Function*, ed. D. E. Bliss, H. L. Atwood and D. C. Sandeman, pp. 185-204. New York: Academic Press.

Govind, C. K. (1992) Age-related remodeling of lobster neuromuscular terminals. *Exp. Gerontol.*, **27**, 63-74.

Govind, C. K. and Walrond, J. P. (1989) Structural plasticity at crustacean neuromuscular synapses. *J. Neurobiol.*, **20**, 409-421.

Govind, C. K., Atwood, H. L. and Lang, F. (1973) Synaptic differentiation in a regenerating crab-limb muscle. *Proc. Natl. Acad. Sci. USA*, **70**, 822-826.

Grinnell, A. D. (1995) Dynamics of nerve–muscle interaction in developing and mature neuromuscular junctions. *Physiol. Rev.*, **75**, 789-834.

Günzel, D., Galler, S. and Rathmayer, W. (1993) Fibre heterogeneity in the closer and opener muscles of crayfish walking legs. *J. Exp. Biol.*, **175**, 267-281.

Hackett, J. T., Cochran, S. L., Greenfield, L. J., Brosius, D. C. and Ueda, T. (1990) Synapsin I injected presynaptically into goldfish Mauthner axons reduces quantal synaptic transmission. *J. Neurophysiol.*, **63**, 701-706.

Halpern, M. E., Chiba, A., Johansen, J. and Keshishian, H. (1991) Growth cone behavior underlying the development of stereotypic synaptic connections in *Drosophila* embryos. *J. Neurosci.*, **11**, 3227-3238.

Hariyama, T., Ozaki, K., Tokunaga, F. and Tsukahara, Y. (1993) Primary structure of crayfish visual pigment deduced from cDNA. *FEBS Lett.*, **315**, 287-292.

Hatt, H. and Smith, D. O. (1976) Non-uniform probabilities of quantal release at the crayfish neuromuscular junction. *J. Physiol. (Lond.)*, **259**, 395-404.

Haydon, P. G., Henderson, E. and Stanley, E. F. (1994) Localization of individual calcium channels at the release face of a presynaptic nerve terminal. *Neuron*, **13**, 1275-1280.

Helfrich-Forster, C. and Homberg, U. (1993) Pigment-dispersing hormone-immunorective neurons in the nervous system of wild-type *Drosophila melanogaster* and of several mutants with altered circadian rhythmicity. *J. Comp. Neurol.*, **337**, 177-190.

Hong, S. J. and Lnenicka, G. A. (1993) Long-term changes in the neuromuscular synapses of a crayfish motoneuron produced by calcium influx. *Brain Res.*, **605**, 121-127.

Hong, S. J. and Lnenicka, G. A. (1995) Activity-dependent reduction in voltage-dependent calcium current in a crayfish motoneuron. *J. Neurosci.*, **15**, 3539-3547.

Hoyle, G. and Wiersma, C. A. G. (1958) Excitation at neuromuscular junctions in Crustacea. *J. Physiol. (Lond.)*, **143**, 403-425.

Iravani, J. (1965) Membrandepolarisation der Muskelfasern des Öffnermuskels des Flusskrebses auf Nervenreiz und Kaliumapplikation. *Experientia*, **XXI**, 609-610.

Kamiya, H. and Zucker, R. S. (1994) Residual Ca^{2+} and short-term synaptic plasticity. *Nature*, **371**, 603-606.

Kang, W.-K. and Naya, Y. (1993) Sequence of the cDNA encoding an actin homolog in the crayfish *Procambarus clarkii*. *Gene*, **133**, 303-304.

Katz, P. S., Kirk, M. D. and Govind, C. K. (1993) Facilitation and depression at different branches of the same motor axon: Evidence for presynaptic differences in release. *J. Neurosci.*, **13**, 3075-3089.

Kennedy, D. and Takeda, K. (1965a) Reflex control of abdominal flexor muscles in the crayfish. I. The twitch system. *J. Exp. Biol.*, **43**, 211-227.

Kennedy, D. and Takeda, K. (1965b) Reflex control of abdominal flexor muscles in crayfish. II. The tonic system. *J. Exp. Biol.*, **43**, 229-246.

Keshishian, H., Chiba, A., Chang, T. N., Halfon, M. S., Harkins, E. W., Jarecki, J., Wang, L., Anderson, M., Cash, S., Halpern, M. E. and Johansen, J. (1993) Cellular mechanisms governing synaptic development in *Drosophila melanogaster*. *J. Neurobiol.*, **24**, 757-787.

Korneev, S., Blackshaw, S. and Davies, J. A. (1994) cDNA libraries from a few neural cells. *Prog. Neurobiol.*, **42**, 339-346.

Kravitz, E. A., Glusman, S., Harris-Warrick, R. M., Livingstone, M. S., Schwarz, T. and Goy, M. F. (1980) Amines and a peptide as neurohormones in lobsters: actions on neuromuscular preparations and preliminary behavioural studies. *J. Exp. Biol.*, **89**, 159-175.

Kravitz, E. A., Schneider, H., Brooks, B. and Eberwine, J. H. (1992) Monitoring gene expression in single neurons from the lobster CNS. *Soc. Neurosci. Abstr.*, **18**, 1413.

Kurdyak, P. A. (1993) Morphological and physiological characterization of neuromuscular junctions in the larva of *Drosophila melanogaster*. M Sc. Thesis, University of Toronto.

Kurdyak, P., Atwood, H. L., Stewart, B. A. and Wu, C.-F. (1994) Differential physiology and morphology of motor axons to ventral longitudinal muscles in larval *Drosophila*. *J. Comp. Neurol.*, **350**, 463-472.

Liang, P. and Pardee, A. B. (1992) Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. *Science*, **257**, 967-971.

Liang, P., Averboukh, L. and Pardee, A. B. (1993) Distribution and cloning of eukaryotic mRNAs by means of differential display: refinements and optimization. *Nucleic Acids Res.*, **21**, 3269-3275.

Llinás, R., McGuinness, T. L., Leonard, C. S., Sugimori, M. and Greengard, P. (1985) Intraterminal injection of synapsin I or calcium/calmodulin-dependent protein kinase II alters neurotransmitter release at the squid giant synapse. *Proc. Natl. Acad. Sci. USA*, **82**, 3035-3039.

Llinás, R., Sugimori, M. and Silver, R. B. (1992) Microdomains of high calcium concentration in a presynaptic terminal. *Science*, **256**, 677-679.

Lnenicka, G. A. and Atwood, H. L. (1985a) Age-dependent long-term adaptation of crayfish phasic motor axon synapses to altered activity. *J. Neurosci.*, **5**, 459-467.

Lnenicka, G. A. and Atwood, H. L. (1985b) Long-term facilitation and long-term adaptation at synapses of a crayfish phasic motoneuron. *J. Neurobiol.*, **16**, 97-110.

Lnenicka, G. A. and Atwood, H. L. (1988) Long-term changes in neuromuscular synapses with altered sensory input to a crayfish motoneuron. *Exp. Neurol.*, **100**, 437-447.

Lnenicka, G. A., Atwood, H. L. and Marin, L. (1986) Morphological transformation of synaptic terminals of a phasic motoneuron by long-term tonic stimulation. *J. Neurosci.*, **6**, 2252-2258.

Luck, A., D'Haese, J. and Hinssen, H. (1995) A gelsolin-related protein from lobster muscle: cloning, sequence analysis and expression. *Biochem. J.*, **305**, 767-775.

Mackler, S. A., Brooks, B. P. and Eberwine, J. H. (1992) Stimulus-induced coordinate changes in mRNA abundance in single postsynaptic hippocampal CA1 neurons. *Neuron*, **9**, 539-548.

Manzanares, M., Marco, R. and Garesse, R. (1993) Genomic organization and developmental pattern of expression of the engrailed gene from the brine shrimp *Artemia*. *Development*, **118**, 1209-1219.

Mercier, A. J. and Atwood, H. L. (1989) Long-term adaptation of a phasic extensor motoneurone in crayfish. *J. Exp. Biol.*, **145**, 9-22.

Monyer, H. and Lambolez, B. (1995) Molecular biology and physiology at the single-cell level. *Curr. Opin. Neurobiol.*, **5**, 382-387.

Msghina, M., Charlton, M. P. and Atwood, H. L. (1995) Differentiation of transmitter release properties and calcium transients in phasic and tonic motor nerve endings of crustacea. *Soc. Neurosci. Abstr.*, **21**, 138.15.

Mulkey, R. M. and Zucker, R. S. (1992) Posttetanic potentiation at the crayfish neuromuscular junction is dependent on both intracellular calcium and sodium ion accumulation. *J. Neurosci.*, **12**, 4327-4336.

Nguyen, P. V. and Atwood, H. L. (1990) Expression of long-term adaptation of synaptic transmission requires a critical period of protein synthesis. *J. Neurosci.*, **10**, 1099-1109.

Nguyen, P. V. and Atwood, H. L. (1992a) Electrical stimulation reprograms the metabolic and synaptic phenotypes of crayfish motoneurons. *Soc. Neurosci. Abstr.*, **18**, 1131.

Nguyen, P. V. and Atwood, H. L. (1992b) Maintenance of long-term adaptation of synaptic transmission requires axonal transport following induction in an identifed crayfish motoneuron. *Exp. Neurol.*, **115**, 414-422.

Nguyen, P. V. and Atwood, H. L. (1994) Altered impulse activity modifies synaptic physiology and mitochondria in crayfish phasic motor neurons. *J. Neurophysiol.*, **72**, 2944-2955.

Ogonowski, M.M., Lang, F. and Govind, C. K. (1980) Histochemistry of lobster claw-closer muscles during development. *J. Exp. Zool.*, **213**, 359-367.

Okimoto, R. and Wolstenholme, D. R. (1993) A tRNA(Ser)(UCN) gene in *Artemia salina* mitochondrial DNA: a case of mistaken identity. *Curr. Genet.*, **24**, 313-315.

Parnas, I. and Atwood, H. L. (1966) Phasic and tonic neuromuscular systems in the abdominal flexor muscles of the crayfish and rock lobster. *Comp. Biochem. Physiol.*, **18**, 701-723.

Parnas, I., Dudel, J. and Parnas, H. (1984) Depolarization dependence of the kinetics of phasic transmitter release at the crayfish neuromuscular junction. *Neurosci. Lett.*, **50**, 157-162.

Pekhletski, R., Cooper, R. L., Pekhletskaia, E., Hampson, D. R. and Atwood, H. L. (1995) Differential dispaly of gene expression in single identified neurons showing long term adaptation due to increased electrical activity. *Soc. Neurosci. Abstr.*, **21**, 72.9.

Pekhletsky, R., Cooper, R. L., Hampson, D. and Atwood, H. L. (1994) Changes in crayfish mRNA expression during motor neuron adaptation. *Amer. Zool.*, **34**, 66A.

Pelliccia, F., Di Castro, M., Lanza, V., Volpi, E. V. and Rocchi, A. (1991) GATA repeats in the genome of *Asellus aquaticus* (Crustacea, Isopoda). *Chromosoma*, **100**, 152-155.

Pumplin, D. W., Reese, T. S. and Llinas, R. (1981) Are the presynaptic active zone particles the calcium channels? *Proc. Natl. Acad. Sci. USA*, **78**, 7210-7213.

Ramon Valverde, J., Batuecas, B., Moratilla, C., Marco, R. and Garesse, R. (1994) The complete mitochondrial DNA sequence of the crustacean *Artemia franciscana*. *J. Mol. Evol.*, **39**, 400-408.

Rheuben, M. B. (1985) Quantitative comparison of the structural features of slow and fast neuromuscular junctions in *Manduca*. *J. Neurosci.*, **5**, 1704-1716.

Robitaille, R., Adler, E. M. and Charlton, M. P. (1990) Strategic location of calcium channels at transmitter release sites of frog neuromuscular synapses. *Neuron*, **5**, 773-779.

Robitaille, R., Garcia, M. L., Kaczorowski, G. J. and Charlton, M. P. (1993) Functional colocalization of calcium and calcium-gated potassium channels in control of transmitter release. *Neuron*, **11**, 645-655.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) *Molecular Cloning*, 2nd ed. New York: Cold Spring Harbor Laboratory Press.

Sherman, R. G. and Atwood, H. L. (1971) Synaptic facilitation: long-term neuromuscular facilitation in crustaceans. *Science*, **171**, 1248-1250.

Shupliakov, O., Atwood, H. L., Ottersen, O. P., Storm-Mathisen, J. and Brodin, L. (1995) Presynaptic glutamate levels in tonic and phasic motor axons correlate with properties of synaptic release. *J. Neurosci.*, **15**, 7168-7180.

Smith, B. R., Wojtowicz, J. M. and Atwood, H. L. (1991) Maximum likelihood estimation of non-uniform transmitter release probabilities at the crayfish neuromuscular junction. *J. Theor. Biol.*, **150**, 457-472.

Smith, S. J., Buchanan, J., Osses, L. R., Charlton, M. P. and Augustine, G. J. (1993) The spatial distribution of calcium signals in squid presynaptic terminals. *J. Physiol. (Lond.)*, **472**, 573-593.

Sotelo, C. (1978) Purkinje cell ontogeny: formation and maintenance of spines. *Prog. Brain Res.*, **48**, 149-170.

Stewart, B. A. and Atwood, H. L. (1992) Synaptic plasticity in a regenerated crayfish phasic motoneuron. *J. Neurobiol.*, **23**, 881-889.

Sucher, N. J. and Deitcher, D. L. (1995) PCR and patch-clamp analysis of single neurons. *Neuron*, **14**, 1095-1100.

Swain, J. E., Robitaille, R., Dass, G. R. and Charlton, M. P. (1991) Phosphatases modulate transmission and serotonin facilitation at synapses: studies with the inhibitor okadaic acid. *J. Neurobiol.*, **22**, 855-864.

Van Gelder, R. N., von Zastrow, M. E., Yool, A., Dement, W. C., Barchas, J. D. and Eberwine, J. H. (1990) Amplified RNA synthesized from limited quantities of heterogeneous cDNA. *Proc. Natl. Acad. Sci. USA*, **87**, 1663-1667.

Van Raay, T. J. and Crease, T. J. (1994) Partial mitochondrial DNA sequence of the crustacean *Daphnia pulex*. *Curr. Genet.*, **25**, 66-72.

Walrond, J. P. and Reese, T. S. (1985) Structure of axon terminals and active zones at synapses on lizard twitch and tonic muscle fibers. *J. Neurosci.*, **5**, 1118-1131.

Walrond, J. P., Govind, C. K. and Heustis, S. (1993) Two structural adaptations for regulating transmitter release at lobster neuromuscular synapses. *J. Neurosci.*, **13**, 4831-4845.

Wernig, A. (1972) Changes in statistical parameters during facilitation at the crayfish neuromuscular junction. *J. Physiol. (Lond.)*, **226**, 751-759.

Wernig, A. (1975) Estimates of statistical release parameters from crayfish and frog neuromuscular junctions. *J. Physiol. (Lond.)*, **244**, 207-221.

Wiersma, C. A. G. (1961) The neuromuscular system. In *The Physiology of Crustacea, Vol. 2*, ed. T. H. Waterman, pp. 191-240. New York: Academic Press.

Winslow, J. L., Duffy, S. N. and Charlton, M. P. (1994) Homosynaptic facilitation of transmitter release in crayfish is not affected by mobile calcium chelators: implications for the residual ionized calcium hypothesis from electrophysiological and computational analyses. *J. Neurophysiol.*, **72**, 1769-1793.

Wojtowicz, J. M. and Atwood, H. L. (1986) Long-term facilitation alters transmitter releasing properties at the crayfish neuromuscular junction. *J. Neurophysiol.*, **55**, 484-498.

Wojtowicz, J. M. and Atwood, H. L. (1988) Presynaptic long-term facilitation at the crayfish neuromuscular junction: voltage-dependent and ion-dependent phases. *J. Neurosci.*, **8**, 4667-4674.

Wojtowicz, J. M., Parnas, I., Parnas, H. and Atwood, H. L. (1988) Long-term facilitation of synaptic transmission demonstrated with macro-patch recording at the crayfish neuromuscular junction. *Neurosci. Lett.*, **90**, 152-158.

Wojtowicz, J. M., Marin, L. and Atwood, H. L. (1989) Synaptic restructuring during long-term facilitation at the crayfish neuromuscular junction. *Can. J. Physiol. Pharmacol.*, **67**, 167-171.

Wojtowicz, J. M., Marin, L. and Atwood, H. L. (1994) Activity-induced changes in synaptic release sites at the crayfish neuromuscular junction. *J. Neurosci.*, **14**, 3688-3702.

Zucker, R. S. (1974a) Crayfish neuromuscular facilitation activated by constant presynaptic action potentials and depolarizing pulses. *J. Physiol. (Lond.)*, **241**, 69-89.

Zucker, R.S. (1974b) Excitability changes in crayfish motor neurone terminals. *J. Physiol. (Lond.)*, **241**, 111-126.

Zucker, R. S. and Bruner, J. (1977) Long-lasting depression and the depletion hypothesis at crayfish neuromuscular junctions. *J. Comp. Physiol.*, **121**, 223-240.

Zucker, R. S. and Lara-Estrella, L. O. (1983) Post-tetanic decay of evoked and spontaneous transmitter release and a residual-calcium model of synaptic facilitation at crayfish neuromuscular junctions. *J. Gen. Physiol.*, **81**, 355-372.

Zucker, R. S., Delaney, K. R., Mulkey, R. and Tank, D. W. (1991) Presynaptic calcium in transmitter release and posttetanic potentiation. *Ann. NY Acad. Sci.*, **635**, 191-207.