Crustacean Phasic and Tonic Motor Neurons

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SYNOPSIS. Crustacean motor neurons subserving locomotion are specialized for the type of activity in which they normally participate. Neurons responsible for maintained activity (‘tonic’ neurons) support moderate to high frequencies of nerve impulses intermittently or continuously during locomotion, while those recruited for short-lasting rapid responses (‘phasic’ neurons) generally fire a few impulses in a rapid burst during rapid locomotion and are otherwise silent. The synaptic responses of the two types, recorded at their respective neuromuscular junctions, differ enormously: phasic neurons exhibit much higher quantal release per synapse and per muscle fibre, along with more rapid synaptic depression and less short-term facilitation. We have analyzed the factors that are responsible for the large difference in initial release of neurotransmitter. Several possibilities, including synapse and active zone size differences, entry of calcium at active zones, and immediately releasable vesicle pools, could not account for the large phasic-tonic difference in initial transmitter output. The most likely feature that differentiates synaptic release is the sensitivity of the exocytotic machinery to intracellular calcium. Molecular features of the phasic and tonic presynaptic nerve terminals are currently under investigation.

INTRODUCTION

Differentiation of motor responses in crustacean limb muscles has been known for almost a century: Lucas, (1907, 1917) observed that two types of contraction could be produced in lobster and crayfish claw closer muscles, apparently resulting from stimulation of two different motor nerve elements. One type of contraction was rapid and twitch-like, while the other was much slower and involved summation of the responses of individual stimuli. Definitive proof of the existence of two types of motor neuron was obtained later by van Harreveld and Wiersma (1937) who were able to isolate individual motor axons supplying the crayfish claw. They found that claw contraction was governed by three individual axons, one inhibitory and two excitatory. The two excitatory axons were termed “fast” and “slow,” according to the type of contraction they produced. Detailed work on a series of isolated crustacean limb muscle preparations showed a consistent pattern of innervation among decapod crustaceans. Several limb muscles have this type of dual innervation—in particular, the closer (adductor of the dactylopodite), bender (flexor of the propodite) and main extensor (carpopodite extensor). Two muscles—the opener (abductor of the dactylopodite) and stretcher (extensor of the propodite)—share a single motor axon which is similar in its physiological properties to the “slow” axons of the dually innervated muscles (Wiersma, 1961).

The duality of the contractions of doubly innervated limb muscles was found to depend on two major features: the synaptic properties of the two excitatory motor neurons (Hoyle and Wiersma, 1958), and the properties of the muscle fibres (electrical and contractile) innervated by these neurons (Atwood, 1963, 1965). Within a single muscle, diverse muscle fibres often occur; the “fast” excitor selectively innervates those that produce action potentials and rapid contractions, while the “slow” axon supplies more extensive innervation to muscle fibres that generate slow contractions activated by repetitive nerve impulses. Many complexities of muscle fibre types and neuromuscular responses have been uncovered, especially in large muscles such as the leg and claw closer muscles of large crabs (Atwood et al., 1965) and the American lobster (Costello and Govind, 1983). Despite these complexities, a clear distinction between the “fast” and “slow” motor axons remains, especially with regard to the synaptic potentials they evoke in their innervated target muscle fibres (Bradacs et al., 1997).

The situation is somewhat different for crustacean abdominal muscles, which were analyzed in the 1960s by Kennedy and Takeda (1965a, b) and Parnas and Atwood (1966). Clear separation of both muscle fibres and motor neurons into parallel twitch (phasic) and slow (tonic) neuromuscular systems was observed. The differences in muscle fibre contraction speed and in excitatory postsynaptic potentials (EPSPs) indicated clear functional separation of the two systems. The motor neurons supplying the twitch muscles are silent except when they are recruited for escape responses (tail flip). In contrast, most of the motor neurons to the slow muscles were spontaneously active in isolated abdomen preparations, responding readily to abdominal sensory inputs with altered impulse discharge (Kennedy and Takeda, 1965a, b).

Crustacean motor systems, in which phasic-tonic differentiation was first established, continue to provide major advantages for experimental investigation of the cellular properties underlying synaptic and neuronal specialization. In this review, we emphasize phasic-tonic synaptic differences and recent experimental tests of the physiological bases of these differences.

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CRUSTACEAN PHASIC AND TONIC MOTOR NEURONS

Differentiation of crustacean motor neurons plays an important behavioural role. This was well established through analysis of reflexes in crayfish abdominal musculature. Many of the tonic motor neurons supplying the slow flexor and extensor muscles are spontaneously active during locomotion, although not when the animal is quiescent (Edwards, 1984). The five different excitatory motor neurons of the slow flexor muscles exhibit different levels of spontaneous activity, and are differentially modulated by discrete tactile inputs (Kennedy and Takeda, 1965a; Page, 1982). In contrast, motor neurons of the phasic flexor and extensor muscles are silent, both in isolated preparations and in the intact animal; they are recruited only for brief rapid actions (escape responses) by massive sensory input acting through giant and non-giant interneurons (Kennedy and Takeda, 1965b; Wine and Krasne, 1982).

Among the motor neurons innervating limb muscles, the same difference in normal impulse activity is apparent: most of the locomotory activity is generated by the tonic (slow) neurons (Bradacs et al., 1997; Lnenicka and Atwood, 1985a, b; Pahapill et al., 1985). Thus, the normally occurring impulse patterns are distinct for well-differentiated phasic and tonic motor neurons, and the difference in efferent impulse delivery is essential for the normal repertoire of locomotion.

The efferent synapses of the motor neurons are adaptively matched to their normal patterns of activity. Indeed, considerable alterations in synaptic properties can be produced simply by altering the motor neuron’s ongoing activity in situ (Lnenicka and Atwood, 1985a, b; 1988).

The underlying causes of the different impulse patterns reside to a large extent in the motor neurons themselves. Analysis of several small systems of crustacean motor neurons has led to the general conclusion that the motor neurons have a central role in formation of efferent output discharges (Wiens, 1982). In part, the central connections among synergistic and antagonistic motor neurons help to produce the necessary patterned discharges. The extensively analyzed circuits of the stomatogastric ganglion illustrate this feature dramatically. Here, the majority of the components producing the rhythmic discharges are the motor neurons, which however are driven to produce different patterns of activity by both sensory and descending central inputs (Combes et al., 2002; Weigeldt et al., 2002; Heinz et al., 2002). Among motor neurons controlling limb and abdominal movements, interactions among motor neurons are also prevalent (Tatton and Sokolove, 1975). For example, the fast (phasic) and slow (tonic) excitatory motor neurons of the crayfish claw closer muscle, and the inhibitory neuron to the claw opener, are mutually coupled by central excitatory connection, which promote their functional synergy (Wiens, 1976; Wiens and Atwood, 1978).

The other important general features of the motor neurons which fit them for specialized production of impulses are their intrinsic membrane properties and their micro-anatomy. Unfortunately, a thorough comparative analysis of the features responsible for phasic-tonic differentiation has yet to be done. The most thorough analysis of neuronal properties for crustaceans is that of the motor neurons of the stomatogastric ganglion, most of which are essential elements for oscillatory rhythm production; these neurons do not readily fit the phasic-tonic classification that has been applied to limb and abdominal motor neurons. Much less detailed information is available for an explanation of the phasic-tonic differentiation of the limb and abdominal motor neurons.

Anatomical features—size and neuronal geometry—have been proposed to make an important contribution to a neuron’s ability to produce impulses in response to central synaptic input. The “size principle” developed for vertebrate central neurons (Henneman et al., 1965) appears to be applicable also to some, at least, of the crustacean motor neurons: tonically active motor neurons of swimmeret and abdominal muscles are generally smaller (Davis, 1971) and endowed with more extensive dendritic ramifications in ganglionic neuropil (Leise et al., 1986) than their phasic counterparts. In addition, there is an inherent phasic-tonic difference in axonal growth: phasic neurons grow more rapidly from cultured explanted abdominal ganglia (Arcaro and Lnenicka, 1995). However, the anatomical differentiation does not hold up very well among limb motor neurons. The phasic and tonic crayfish claw closer neurons have similar sized somata and parallel dendritic branching (Wiens, 1976, 1982). The phasic axon of the crayfish limb extensor is actually smaller than its tonic counterpart (Bradacs et al., 1997). In snapping shrimp, no difference in morphology and passive electrical properties could be found for the claw closer motor neurons which supply the dimorphic pincer and snapper closer muscles and which produce very different efferent impulse discharges (Wilson and Mellon, 1982). Such observations support the conclusion that anatomical features in themselves cannot account for differences in impulse patterns, especially among limb motor neurons. This conclusion is reinforced by the fact that phasic and tonic closer motor neurons, driven by the same synaptic inputs, produce very different outputs (Wiens and Atwood, 1978).

Long-standing evidence from isolated axons confirms the existence of differential responsiveness to applied currents (Wright and Adelman, 1954; Wright and Coleman, 1954). Impulse discharges of phasic axons accommodate more rapidly and thus produce only short bursts for a maintained stimulus, while more tonic axons produce long-lasting discharges. The differential occurrence of potassium channels giving I\textsubscript{A}, current, and differences in Na\textsuperscript{+} channel inactivation, have been implicated (Connor, 1975; Atwood, 1982).

More detailed descriptions of motor neuron mem-
brane channels have emerged from studies of fast (phasic) flexor neurons, and also from work on swimmeret neurons (Chrachri, 1995). The somata of fast flexor motor neurons usually do not produce action potentials, but nevertheless possess Na and Ca channels which can generate two types of action potential when K channels are partially inactivated, or when Na channel inactivation is alleviated (Czternasty et al., 1984, 1989; Roux-Bruxelle et al., 1991; Bruner et al., 1986). In fast flexor motor neuron F3, two types of Ca channel have been described, one resembling P/Q type channels of other organisms, and another less readily characterized (Hong and Lnenicka, 1995, 1997).

The P/Q-type Ca channel is thought to occur also at synaptic terminals and to be the most important one for neurotransmitter release (Hong and Lnenicka, 1997; Araque et al., 1994). However, additional N-type and R-type Ca channels have been reported at phasic and tonic crab neuromuscular junctions, respectively (Rathmayer et al., 2002), indicating the possibility of calcium of channel differentiation in phasic and tonic neurons.

The complexities of channel expression and localization have been explored most extensively in the motor neurons of the stomatogastric system, especially for K channels (Baro, 2002). Here, differential expression patterns of the subunits contributing to K channels responsible for the early potassium current (I_A) have been described, together with differential localization in the soma, axon, and synaptic terminal regions. The differences in K channel expression and modulation among the neurons of the pyloric network are thought to impart unique properties that shape the impulse discharge patterns of the neuron (Baro, 2002; Tierney and Harris-Warrick, 1992).

Undoubtedly, this type of differentiation is likely to be important for impulse generation in phasic and tonic motor neurons. Invariably, the more tonic, low-threshold motor neurons of crustacean motor networks have been found to generate trains of spikes much more readily than their phasic counterparts (Takahashi and Takahata, 1995; Sillar and Elson, 1986). Probably the K channels are a major determinant of this difference.

### NEUROMUSCULAR MORPHOLOGY AND TRANSMITTER RELEASE PROPERTIES

#### Nerve terminal ultrastructure

There is a clear difference in nerve terminal morphology between tonic and phasic neurons. A summary of morphological data is shown in Table 1. In the crayfish claw closer (Lnenicka et al., 1986) and in the crayfish leg extensor (King et al., 1996), phasic

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<th>Phasic</th>
<th>Tonic</th>
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<td>Innervation</td>
<td>“Fast” abdominal muscles; excitatory innervation exclusively phasic.</td>
<td>“Slow” abdominal muscles; excitatory innervation exclusively tonic.</td>
<td>Kennedy and Takeda (1965a,b) van Harreveld and Wiersma (1937)</td>
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<td>Long-term plasticity</td>
<td>Low frequency stimulation applied intermittently over several days produces a persisting modification of release (lower initial quantal output, resistance to depression, long-term adaptation). Intermediate frequency stimulation maintained for several minutes produces a potentiation of release (long-term facilitation)</td>
<td>Maintained intermediate frequency stimulation produces a persisting enhancement of transmitter release (long-term facilitation).</td>
<td>Lnenicka and Atwood (1996)</td>
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<td>Synaptic morphology</td>
<td>~1.5 synapses per μm terminal length. ~0.2 μm² synaptic area. ~1.6 active zones per synapse (often in closely spaced pairs). ~2.1 active zones per μm² bouton volume. (Crayfish carpopodite extensor)</td>
<td>~3.43 synapse per μm terminal length. ~0.25 μm² synaptic area. ~1 active zone per synapse. ~0.8 active zones per μm³ bouton volume. (Crayfish carpopodite extensor)</td>
<td>King et al. (1996) Mshghina et al. (1998, 1999)</td>
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terminal boutons were found to be thin and filiform, whereas tonic boutons are larger in diameter and have a varicose appearance; bouton volume was 5 times larger for the tonic neuron. This difference is related to the larger mitochondrial volume of tonic boutons (Lnenicka et al., 1986). Further, the number of both synapses and active zones per length terminal is significantly larger for the tonic axon in the leg extensor muscle (King et al., 1996). Thus, possession of a greater overall number of synapses per bouton does not confer greater synaptic strength (defined as the size of the postsynaptic response produced by a single nerve impulse with low frequency stimulation) to the tonic nerve terminal. In fact, the phasic neuron typically has fewer synapses per bouton than the tonic neuron, yet emits far more quanta per impulse at low frequencies of activation. However, individual active zones were found to be longer on average in phasic terminals, and a higher proportion of phasic synapses had multiple active zones, suggesting that synaptic complexity and size could contribute to synaptic strength (Cooper et al., 1996a, b; King et al., 1996). Freeze-fracture replica analysis at the crayfish leg extensor motor neuron synapses showed that both types of synapse contain on average approximately 15 large active zone particles, which are generally thought to be mostly voltage gated calcium channels (Msghina et al., 1999). Overall, results to date point to physiological rather than morphological differences at the individual synapse making the major contribution to synaptic strength. In addition, the number and length of nerve terminal branches on a muscle fibre is usually greater for phasic motor neurons (Brown and Newby, 1980) and this feature contributes to the large quantal content per muscle fibre and large EPSPs characteristic of phasic motor neurons as a group.

Transmitter release properties

A difference in synaptic strength has long been observed in phasic and tonic neurons (see Table 1 for a summary of general transmitter release properties). In all preparations such as the deep abdominal flexor, deep abdominal extensor, and leg extensor muscles of crayfish, activation of a phasic neuron produces a relatively large excitatory postsynaptic potential (EPSP) in the innervated muscle fibre, which often generates a muscle action potential. Repeated stimulation even at low frequency results in depression of the EPSP (Kennedy and Takeda, 1965b; Parnas and Atwood, 1966; Bradacs et al., 1997). Conversely, stimulation of a tonic neuron in preparations such as the superficial abdominal flexors or extensors, the opener, or the leg extensor, produces a much smaller EPSP. Further, repeated stimulation produces a marked frequency-dependent facilitation of the EPSP (Kennedy and Takeda, 1965a; Dudel and Kuffer, 1961a; Bradacs et al., 1997).

Extracellular recording microelectrodes permitted the examination of quantal properties of release: the number of individual quanta released from a small subset of synapses located on a single synaptic bouton can be measured. Recordings from phasic boutons showed that when stimulated at low frequencies, these neurons always produced transmitter release that was multi-quantal; that is, the size of the evoked current was many times the size of spontaneously occurring ones thought to represent individual quanta (Atwood, 1967; Brown and Newby, 1980; Msghina et al., 1998, 1999; Dudel and Kuffer, 1961b; Bradacs et al., 1997). In the crayfish leg extensor preparation, the number of quanta released per action potential (quantal content) was approximately 15, at low frequencies of stimulation (Millar et al., 2002). In stark contrast, when extracellular recordings were made from tonic boutons of preparations such as the crayfish opener or main leg extensor, low frequency stimulation often resulted in one or no quanta being released from the bouton (Wojtowicz et al., 1994; Cooper et al., 1995b, 1996a; Msghina et al., 1998, 1999; Dudel and Kuffer, 1961b; Bradacs et al., 1997). Recent work at the leg extensor muscle showed that quantal content at tonic boutons was less than 0.05, indicating that for every 100 stimuli applied, only 5 quanta on average were released (Millar et al., 2002) (see Fig. 1).

These results show clearly the extreme difference in the overall probability of initial transmitter release from crustacean tonic and phasic nerve terminals. In fact, Msghina et al. (1999) showed that at the leg extensor, the amount of release per synapse is 100–1000 times greater for the phasic neuron.
calcium entry and clearance; measured value represents the balance between entry and extrusion, as detailed in the single-compartment model of Tank et al. (1995).

First, the calcium removal rate will have a large affect on the measured peak level of \([\mathrm{Ca}^{2+}]\), (Tank et al., 1995). In simple terms, the more rapidly calcium is removed from the terminal, the lower the peak calcium. It was found that calcium removal rate was twice as rapid at tonic boutons, thus reducing their peak \([\mathrm{Ca}^{2+}]\), levels in comparison to those of phasic boutons.

Second, the number of synaptic active zones per volume of terminal will also affect the peak level of \([\mathrm{Ca}^{2+}]\). Since synaptic active zones are believed to be the major calcium entry point during stimulation (Deplaney et al., 1988; Tank et al., 1995), a greater number of active zones per unit of bouton volume will result in a greater amount of measured \(\mathrm{Ca}^{2+}\) accumulation. Ultrastructural examination of phasic and tonic nerve terminals has shown that phasic terminals do in fact contain 2–3 times more active zones per um\(^3\) of bouton volume. This difference in itself would result in a larger whole-bouton calcium accumulation.

Calcium entry at phasic and tonic synapses can be compared by normalizing the calcium accumulation values for both calcium removal rate and the number of active zones per unit volume of cytoplasm. With these corrections, calcium accumulation values are very similar on average for the two terminal types. Given that the corrected accumulation values are similar, calcium entry per active zone (and per synapse), which leads to accumulations, must also be comparable. Any remaining difference in calcium entry per synapse could not be large enough to cause a 100–1000 fold difference in transmitter release. Thus, this hypothesis, based on differential calcium release at the two types of synapse, was falsified.

Readily releasable vesicles

Synaptic vesicles that can be released immediately upon stimulation without mobilization to the active zone are often termed readily releasable vesicles (RRVs). This class of vesicles is thought to be analogous to morphologically docked vesicles, which can be seen physically touching the presynaptic active zone in electron micrographs (Schikorski and Stevens, 1999). Several recent studies of cultured mammalian

SYNAPTIC MECHANISMS

The drastic difference in the amount of transmitter released from phasic and tonic synapses implies a pre-synaptic mechanism which cannot be explained by differences in bouton or synaptic ultrastructure. It is natural to pose the question: What are the mechanisms that govern a thousand-fold difference in transmitter release? There are three synaptic mechanisms which might account for the observed difference: 1) The calcium concentration at the transmitter release site; 2) The number of vesicles readily available for release; and 3) The sensitivity of the transmitter release mechanism to calcium. These mechanisms have been examined largely in the phasic and tonic motor neurons of the crayfish carpopodite extensor muscle; thus, this section will focus primarily on results from these neurons (see Table 2 for a summary of results).

Release site calcium concentration

In all synapses, neurotransmitter release is initiated by an elevation in calcium concentration at the release site (Katz, 1969). Furthermore, the amount of transmitter released increases logarithmically with the calcium concentration (Dodge and Rahamimoff, 1967; Augustine et al., 1985). Work at the crayfish opener preparation showed that calcium accumulation was higher in boutons releasing greater amounts of transmitter (Cooper et al., 1995a). Thus, one could hypothesize that calcium entry and therefore calcium concentration reached at release sites of phasic synapses is greater than for tonic synapses, resulting in more transmitter release (see Fig. 2A).

This hypothesis was tested by Msghina et al. (1999), by comparing \([\mathrm{Ca}^{2+}]\) during stimulation at both phasic and tonic nerve terminals. Using the calcium indicator Fura-2, whole-bouton calcium accumulation during short stimulus trains was measured in both terminal types. It was found that in phasic terminal boutons, calcium accumulated to levels five-fold higher than in tonic boutons. On first inspection, this result seemed to support the initial hypothesis of greater calcium entry per synapse in phasic boutons. However, several factors affect the overall cytoplasmic calcium accumulation in the bouton, as measured by Fura-2. The measured value represents the balance between entry

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<td>Readily releasable and docked vesicles</td>
<td>–50 readily releasable vesicles per bouton. (Crayfish carpopodite extensor)</td>
<td>–130 readily releasable vesicles per bouton. (Crayfish carpopodite extensor)</td>
<td>Millar et al. (2002)</td>
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<td>Calcium entry and clearance</td>
<td>–5-fold greater ([\mathrm{Ca}^{2+}]), accumulation than for tonic (bouton volume is smaller).</td>
<td>–5-fold smaller ([\mathrm{Ca}^{2+}]), accumulation than for phasic (bouton volume is larger).</td>
<td>Msghina et al. (1999)</td>
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<td>–2-fold slower ([\mathrm{Ca}^{2+}]), removal than for tonic boutons. (Crayfish carpopodite extensor)</td>
<td>–2-fold more rapid ([\mathrm{Ca}^{2+}]), clearance than for phasic boutons. (Crayfish carpopodite extensor)</td>
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TABLE 2. Synaptic Mechanisms.
neurons have shown that the amount of transmitter released initially is directly proportional to the size of the readily releasable pool (RRP) of synaptic vesicles (Stevens and Tsujimoto, 1995; Rosenmund and Stevens, 1996; Dobrunz and Stevens, 1997; Schikorski and Stevens, 1999, 2001). Millar et al. (2002) proposed the hypothesis that phasic synapses possess a larger RRP than tonic synapses, resulting in greater initial transmitter release (see Fig. 2B).

To test this hypothesis, a measurement of RRP sizes was required. A rapid vesicle depletion technique, employed by Schneggenburger et al. (1999), was used to obtain a comparative measure of RRP. During a rapid train of stimuli, the RRP is thought to be depleted, and transmitter release is accordingly depressed. One can measure the number of quanta released during this initial depression, and factor out any remaining release due to pool replenishment. This gives a measure of vesicles which were readily releasable before pool replenishment.

This type of analysis was performed at both phasic and tonic terminal boutons, under conditions which promoted rapid depletion of the RRP. Contrary to the initial hypothesis, it was found that tonic boutons contained more RRVs than phasic boutons (on average, ~130 RRVs for tonic boutons, compared to an average

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**Fig. 2.** Possible mechanisms governing large presynaptic release differences (RRV, readily releasable vesicle; AP, action potential; NT, neurotransmitter; PSP, postsynaptic potential; Ca^{2+}, local calcium ion microdomain). A: Synaptic Calcium Entry. In this mechanism, paired phasic and tonic nerve terminals contain similar numbers of readily releasable vesicles, and have similar calcium sensitivities of transmitter release; however, the amount of calcium entering at the synapse is greater at the phasic terminal. The hypothesis is that more calcium entry results in a higher probability that a readily releasable vesicle will be exocytosed, and thus in a greater number of vesicles contributing to the PSP. B: Readily Releasable Vesicles. In this mechanism, phasic and tonic terminals have similar amounts of calcium entry at the active zone, and similar calcium sensitivities; however, in this case, the number of readily releasable vesicles is greater at the phasic synapse. The hypothesis is that for a given amount of calcium entering at the synapse, more vesicles are exocytosed from the phasic terminal, because a greater number of the available vesicles are readily releasable. C: Calcium Sensitivity of Release. In this mechanism, phasic and tonic terminals have similar amounts of synaptic calcium entry and contain similar numbers of readily releasable vesicles; however, the calcium sensitivity of the release process is higher at the phasic synapse (depicted with a larger calcium sensor). The hypothesis is that calcium entry and readily releasable vesicle numbers are similar, and that a greater sensitivity of the release process to calcium entry at phasic synapses results in a greater probability of vesicle exocytosis, and in a larger number of vesicles released per terminal, leading to a larger PSP.
of ~60 RRVs for phasic boutons; see Fig. 3). A comparison of the normal number of vesicles (quanta) released during a single action potential, to the total number of vesicles readily available for release (RRP), gives the “released fraction” of total available vesicles released by an action potential. This is a direct measure of individual vesicle release probability. It was calculated that the released fraction for phasic synapses was ~30%, and for tonic synapses ~0.02%; this represents a 1500-fold difference in release probability (Millar et al., 2002).

Differences in RRP sizes were confirmed with ultrastructural analyses. Docked vesicles were counted at synapses which had been serially reconstructed. In agreement with RRP results, tonic synapses contained on average ~11 docked vesicles, whereas phasic synapses contained on average ~4 docked vesicles. Therefore, the hypothesis that phasic synapses contain a larger number of RRVs or docked vesicles, and that such a difference is the cause of a 1500-fold difference in release probability, was not supported by this study.

Calcium sensitivity of release

A final mechanism which could govern such a drastic difference in transmitter release probability is the sensitivity of the vesicular release process to the calcium concentration reached at the release site. Past studies have suggested widely varying calcium sensitivities among different neurons. At goldfish retinal bipolar cells, the calcium requirement for physiological release rates is reported to be >100 μM (Heidelberger et al., 1994). In contrast, studies of cells in the rat auditory brainstem suggest that these neurons have a much higher calcium sensitivity of release, requiring ~10 μM to produce physiological release rates. It is possible that a difference in calcium sensitivity exists at phasic and tonic synapses, such that transmitter release at tonic synapses has a higher sensitivity to calcium, producing greater amounts of transmitter release with similar amounts of calcium entry (see Fig. 2C). The evidence suggests that there could be a difference at the level of individual vesicle exocytosis, which could be produced by molecular differences in the calcium sensor triggering release.

DISCUSSION

The principles of organization of motor systems in decapod crustaceans, and in particular the differentiation of components (motor neurons and muscle fibers) into sets specialized for rapid movement and sets specialized for slow movement or postural adjustments, are found in many other phyla. However, details of specialization vary amongst different groups of organisms. In decapod crustaceans, the small number of motor neurons supplying each muscle, and the large size of the muscle fibers, has allowed clear definition of the peripheral motor systems. In vertebrates, there are many more motor neurons for each muscle, so that consistent identification of individual motor neurons is less feasible. However, much work has been done on the properties of vertebrate motor units (each comprising a motor neuron and its group of innervated muscle fibers); “glycolytic” and “oxidative” muscle fibers correspond functionally with the “phasic” and “tonic” motor complexes of decapod crustaceans. Thus, specialized motor systems with phasic-tonic differentiation have evolved in many groups of successful organisms.

The unambiguous identification of individual motor neurons in decapod crustaceans provides a particularly favourable situation for investigating synaptic differences. Evolution of synapses differing dramatically in physiological properties has contributed a major element to the specialized response pathways subserving fast and slow actions. The very large differences in transmitter release at boutons of phasic and tonic motor neurons provide an ideal experimental model for analyzing the basis of synaptic differentiation. As indicated in the preceding analysis, it appears likely that molecular differences in presynaptic terminals governing the exocytotic machinery are important (Atwood and Karunanithi, 2002). This conclusion is derived from a series of experiments in which other possibilities were systematically eliminated. If sustained in further experiments, this feature would explain in large measure the characteristic difference in transmitter release per impulse (synaptic strength).

Another very important difference between phasic and tonic motor neurons emerges from comparison of their rates of synaptic depression (synaptic fatigue) during maintained stimulation; tonic synapses are much more resistant to depression. A difference in mitochondrial content of boutons has been linked to synaptic depression (Nguyen et al., 1997; Atwood et al.,


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