ABSTRACT: We present a model preparation, the crayfish, to investigate chronic stimulation effects in muscle fiber type and neuronal conversion from fast to slow. The results show a presynaptic alteration in transmitter release after 1 week of stimulation at 5 Hz for a 2-h daily regime. With the same stimulation paradigm, the muscle proteins displayed on a polyacrylamide gel only start to show changes after 3 weeks. The original phasic motoneurons within 1 week display an enhanced ability to resist synaptic depression, as do tonic motoneurons. The results show that identified phasic motoneurons and muscle fibers in the crayfish can be transformed to a toniclike state, and that the nerve terminals convert prior to the muscle fibers. Electrophysiological clinical measures indicating a change in transmitter release properties may not necessarily mean that the muscle fibers have fully adapted for long-lasting effects. This preparation allows stimulation conditions to be examined with ease.
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ACTIVITY OF PHASIC MOTOR NEURONS PARTIALLY TRANSFORMS THE NEURONAL AND MUSCLE PHENOTYPE TO A TONIC-LIKE STATE

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Fast and slow skeletal muscle fibers have distinguishing characteristics such as shorter sarcomeres, a reduced thin-to-thick filament ratio, and higher myofibrillar adenosine triphosphatase (ATPase) activity in fast as compared to slow fibers. Some thin filament proteins, such as troponin T, I, and C, show differing characteristics among the fiber types that help in specific muscle identification. In addition to the anatomical and protein differences, there are also general differences in measured electrophysiological responses. Upon repetitive nerve stimulation of tonic muscles, the postsynaptic muscle response is usually fatigue resistant, retaining the amplitude of the depolarizing response and the developed tension; stimulation of neurons innervating phasic muscles commonly shows a depressed depolarizing response and a decreased excitatory postsynaptic potential (EPSP) over a relatively short time period. These structural and physiological characteristics are significantly dependent upon the type of neural innervation (i.e., a phasic or tonic motor neuron). Aside from dependence on the specific neuronal type, there is a strong relationship between the activity pattern of the neuron and maintenance of a particular muscle fiber type.

Neuronal activity is widely accepted as a contributing factor for appropriate neural development in a variety of animal species. The pioneering work of Wiesel and Hubel[47] on the development of the visual system, in the presence and absence of visual stimuli, demonstrated that gross morphological alterations appear in synaptic organization in the various parts of the brain. Heightened synaptic input to a postsynaptic neuron can result in structural, long-lasting changes of a postsynaptic cell, as was shown for Purkinje cells in the cerebellum[46] and hippocampal neurons.[26]

In mammals, when the postsynaptic cell is a muscle fiber, striking biochemical and structural changes occur with alteration of synaptic activity.[16,27,28,45] Likewise in crayfish, the presynaptic pha-

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sic motor nerve terminals that are responsible for synaptic transmission show structural and physiological differences when electrical activity is altered.[31,39] To gain a better understanding of the morphological and physiological changes that occur in single cells as a result of neural activity, simpler preparations have been utilized, including *Aplysia* and crayfish. Long-term adaptations (LTA) that persist for hours or days in presynaptic neurons due to increased electrical activity were shown to occur at the crayfish neuromuscular junction[31] as well as in the central nervous system of *Aplysia,* in which habituation and sensitization occur.[10] The persistent changes that occur in these invertebrate systems were shown to be protein synthesis dependent.[17,39] Blocking protein synthesis prior to the onset of conditioning activity retards the alterations, thus indicating that a critical period is present. Consequently, there is strong indication that gene regulation is also involved.[14,15]

Protein synthesis dependent on increased electrical activity occurring in motor nerve cell bodies is responsible for particular motor terminal changes at the site of transmitter release in crustaceans.[40] As with heightened electrical activity of tonic muscles, there is an increase in muscle mass due to protein synthesis. Crustacean muscle, like mammalian muscle, can be classified into different fiber types based on metabolic activity as well as protein composition.[16,38]

A preparation which provides the optimal conditions to correlate activity with physiological and structural changes is the crustacean neuromuscular junction. There are a number of unique differences between crustacean and vertebrate phasic and tonic motor neurons and the changes that occur during phasic/tonic interconversion. In crustaceans, muscles usually are selectively innervated by either a phasic or a tonic motor neuron, although some single fibers can be innervated by both phasic and tonic excitatory motor neurons. Through selective stimulation of one or the other motor neuron, physiological differences in the EPSPs may be measured. Normally phasic motor neurons are silent, but when active, they can drive rapid twitching of muscle fibers and evoke EPSPs on the order of 10–40 mV. The phasic response depresses rapidly with 5–10-Hz trains of stimulation. Tonic motor neurons, however, have a higher intrinsic activity. They give rise to smaller EPSPs that can be facilitated in the presence of a higher frequency (10–50 Hz) of stimulation.[1,2,40] The tonic muscle contraction is often slower and graded when compared to the phasic response. Structurally, the presynaptic phasic and tonic terminals are different. The phasic terminals are 1–2 μm in diameter and have a thin, filiform shape. Tonic terminals are bigger, have 5–20-μm-diameter varicosities, and bottlenecks between the swellings. Differences in mitochondrial content reflect the varied activity patterns and energy needs of these neurons; tonic neuron mitochondria are larger in cross-sectional area and are also more branched.[30,35]

Transformation to a tonic-like physiological and morphological state can be achieved by electrically conditioning phasic neurons for a few hours daily over 7 days. Persistent change has been shown to occur in two particular motor neurons which innervate distinctly different muscles in crayfish, the claw closer and the abdominal extensor muscles.[31,36] The converted phasic neuron produces a reduced initial EPSP amplitude and fatigue resistance as well as increased facilitation.[8,31,32,34,36] The terminal transformation from thin filiform to varicose is accompanied by an increase in mitochondrial cross-sectional area and branching.[30,35]

The objective of this article is to report on the properties associated with phasic muscle fibers after transformation of neuronal phenotype by electrical conditioning. We hypothesize that, as with motor neurons, there should be transformation in the proteins expressed in the muscle fibers. Such a relatively simple, well-defined in vivo experimental system exhibiting activity-dependent modulation of gene expression should encourage better understanding of the cellular mechanisms regulating transcription. Working with reproducibly identifiable motor neurons and muscle fibers should provide an insight regarding the molecular differences among different types of motor neurons and muscles.[6,7,43] The crayfish neuromuscular junction continues to be used widely as a model preparation to examine the basic mechanisms involved in synaptic transmission.[4,18–23,25,49] Understanding mechanisms of activity-induced regulation of protein synthesis in ''simpler'' preparations, where physiology and ultrastructure can be correlated, provides a working model for the more complex systems in the invertebrate and vertebrate central nervous system (CNS). Findings from the invertebrate research presented here may establish a foundation for future work on vertebrate neuromuscular systems.

METHODS

All experiments were performed using the crayfish, *Procambarus clarkii,* measuring 10–14 cm in body length (Atchafalaya Biological Supply Co., Raceland,

LA). Animals were housed in an aquatic facility and fed dried fish food and carrots *ad libitum*.

The experimental design consists of chronically implanting two platinum wires, 1–2 mm through the cuticle, on either side of the nerve leading to the abdominal extensor muscles in segment three.[36,42] The sham control had platinum wires on the contralateral side to the experimental side within the same segment (Fig. 1). Conditioning of the neurons was accomplished by applying suprathreshold stimulation of 1-s bursts at 5 Hz every 2 s for 2 h per day for 7, 14, or 21 days as previously described.[36]

EMG Response. In order to confirm that the motor neurons are being stimulated, a second set of platinum wires were placed under the dorsal carapace in line with the L1 and M deep extensor muscles (Fig. 1). In this arrangement one can easily monitor the activity of the extensor muscles during the conditioning period.

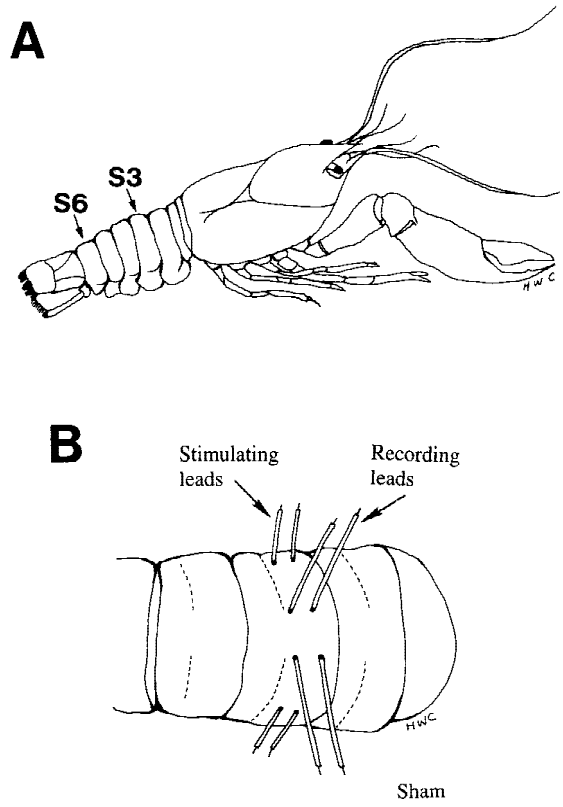

FIGURE 1. Location of the chronic implants for long-term conditioning. **(A)** SIde view of a crayfish. Legs shown only on one side. **(B)** An enlargement of the abdomen illustrating the locations of wires placed to stimulate the dorsal nerve root and to record EMG activity. The stimulating wires are placed so that the nerve root lies between the two leads. The tips of each lead extend approximately 1 mm past the inner surface of the cuticle. The EMG recording leads are also placed approximately 1 mm deep close to the base of the M muscle. S, segment.

EPSP Measures. In order to make intracellular muscle recordings, the ventral surface of the deep extensor was exposed by removing the ventral side of the abdomen after cutting along the length of the abdomen approximately at the midline on each side. The dorsal half was pinned down on a Sylgard-coated dish and residual flexor muscle removed. This allows for excellent visual identification of the deep extensor muscles (L1, L2, and M) and the superficial lateral extensor muscle (SLE). The superficial medial extensor muscle (SME) can be seen after removal of the L1 and L2 musculature (Fig. 2). Dissected preparations were maintained in crayfish saline, a modified Van Harreveld's solution (in mmol/L: 205 NaCl; 5.3 KCl; 13.5 $CaCl_2 \cdot 2H_2O$; 2.45 $MgCl_2 \cdot 6H_2O$; 10 glucose; 0.5 HEPES adjusted to pH 7.4). In order to insure that stimulating the inhibitory axon would not alter the postsynaptic excitatory responses, picrotoxin (1 mmol/L) was used.[9,36] During the dissection, the muscles were bathed in the medium, and it was exchanged every 20 min with a chilled (14°C) replacement until dissections were completed. While stimulating the nerve root in segment 2, responses were measured on the conditioned and sham sides within segment 2 as well as in both sides of the next posterior segment (segment 3). Repeated measurements of intracellular muscular responses within segment 3 were made while stimulating the nerve root in segment 3.

Sodium Dodecyl Sulfate Electrophoresis. The abdomen of the crayfish was dissected leaving the L1, L2, SME, SLE, and M muscles attached to the cuticle. The cuticle and attached muscles were quickly frozen at –20°C for 20 min to cause apolysis.[41] Tissues were removed from the freezer and rinsed twice in cold 0.5 mol/L NaCl and 5 mmol/L sodium phosphate (pH = 7.4). Next, the tissues were glycerinated in cold buffer containing 20 mmol/L Tris–acetate (pH = 7.5), 50% glycerol, 0.1 mol/L KCl, 1 mol/L edetic acid, 0.1% Triton X-100 for 1 h. The L1 and L2 of three abdomen segments were removed and placed into labeled vials containing 50 µL of reagent A [0.34 g of sodium phosphate (anhydrous, monobasic), 1.02 g of sodium phosphate (anhydrous, dibasic), 1.0 g of sodium dodecyl sulfate (SDS), 1 mL 2-mercaptoethanol, 0.0151 g Bromphenol Blue, and 36 g urea]. Care was taken not to remove the two muscle fibers associated with the muscle receptor organs. The M muscles were removed and placed in a labeled vial containing 100 µL of reagent A. The vials were left overnight at room temperature. Prior to electrophoresis, the samples were heated at 90°C for 3 min. Ten percent SDS–polyacrylamide gels

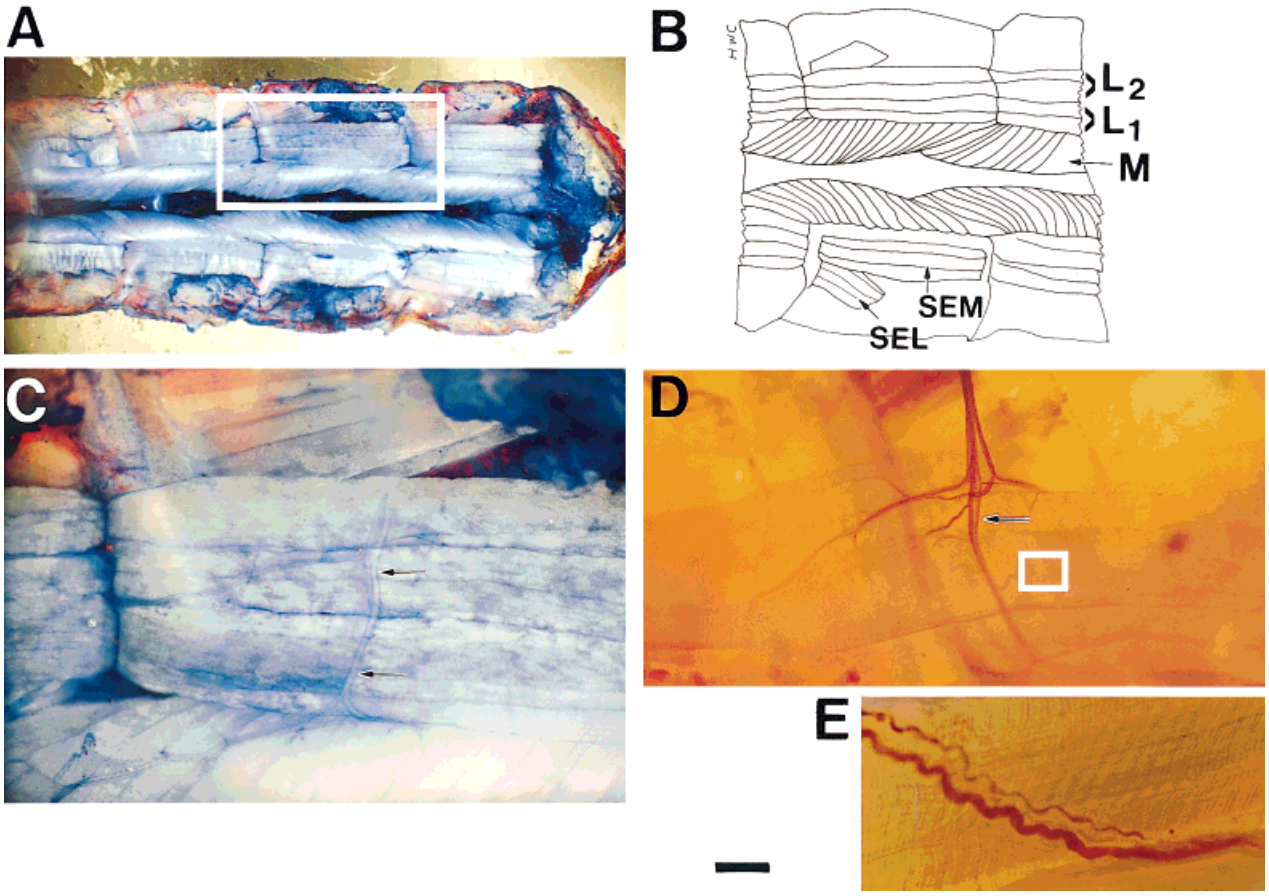

FIGURE 2. The innervation pattern and layout of the deep extensor muscles. **(A)** Viewing the extensor muscles from the ventral side toward the dorsal aspect depicted in Figure 1. **(B)** A schematic of the muscles shown in **(A)** with the particular muscles identified. The most medial muscles are the M muscles, with a spiral fiber pattern. In the top half the deep extensors muscles are shown. L1 is the first lateral group followed by the L2 lateral of the deep extensors. In the bottom half, the L1 and L2 musculature was removed to show the more dorsal superficial extensor muscles. The superficial extensor medial muscle (SEM) lies directly dorsal to L1 and L2. The most lateral bundle of fibers is the superficial extensor lateral muscle (SEL). The M, L1, and L2 are phasic, and the SEM and SEL are tonic in control preparations. **(C)** Methylene blue staining of the deep extensor muscles and nerve bundle (arrows) illustrating the innervation pattern. **(D)** Horseradish peroxidase (HRP) forward filling of the neurons (arrow) innervating the muscles to distinguish specific terminal innervation of the various fibers. Note that two neurons cross from segment 2 to the L1 muscles in segment 3 (arrowheads). Inset shown is magnified below. **(E)** The HRP-filled terminals indicate the slight swelling in the thin phasic terminals innervating L1 (arrows). Scale bar: **A, B,** 0.6 mm; **C, D,** 330 μm; **E,** 10 μm.

were used (30% acrylamide/0.8% bisacrylamide in a 4× Tris-Cl/SDS solution). The running buffer was a glycine buffer (BioRad), containing SDS. Gels were stained with Coomassie Brilliant Blue R250, and following destained gels were digitized.

RESULTS

Conditioning. The procedure used for chronic nerve root stimulation allows one to easily modify the stimulating parameters until an electromyographic (EMG) response is recorded by the differential recording leads (Fig. 1). The innermost cuticular layers grow around the bare ends of the implanted leads. After 1 week the leads have a soft, fibrous tissue covering, and within 3 weeks the soft tissue hardens so that the leads are encased. In these studies no necrosis of the cuticular tissue was observed. The SLE muscles are damaged by the implantation of the stimulating wires, as can be seen in Figure 1. After 1 week the SLE muscles start atrophying, becoming more pronounced by the end of the 3-week conditioning period. This is the only muscle damaged by this procedure. Damage is related to the placement of the leads, one of which goes through the region where the muscle attaches to the cuticle or directly into the muscle itself. The SME and the deeper muscles do not come into contact with the stimulating or the recording leads (Figs. 2A and C).

The methylene blue staining and horseradish peroxidase (HRP) forward filling in Figures 2C and

D reveal the innervation pattern of the deep extensor muscles, which supports the physiological mapping of the postsynaptic responses[9] recorded in these muscles. The fine terminals of the phasic neurons are easily seen with the HRP loading (Fig. 2E).

Monitoring electrical activity in muscles during a regimen of stimulation-induced contraction ensures that the nerve root is being stimulated. During the first few days of such conditioning, the EMGs are maintained for only approximately 40 min of the 2-h stimulation period. Gradually over the next few days, the EMG activity continues for longer periods. By the end of the 3 weeks of continual stimulation the responses were present for the full 2 h of stimulation. These results can be explained by increased fatigue resistance. Since the recording leads are placed closest to the M muscles, the most massive of the extensors, the responses recorded with the EMG during the stimulation train likely result from electrical activity in these initially phasic (M) muscles (Fig. 3A, arrows).

When the animal initiates movement on its own, a number of different-sized spikes in the EMG recording can be seen, indicating that a number of muscles are being recorded. That these various spikes are not observed during the stimulation is likely due to the five pulses given at 5 Hz being insufficient to facilitate tonic responses as compared to the voluntary activity of extending the abdomen. In addition, the large flexor muscles were active during the movements when recording the responses shown in Figure 3B. The responses became more uniform in size following an extension of the abdomen (Fig. 3C).

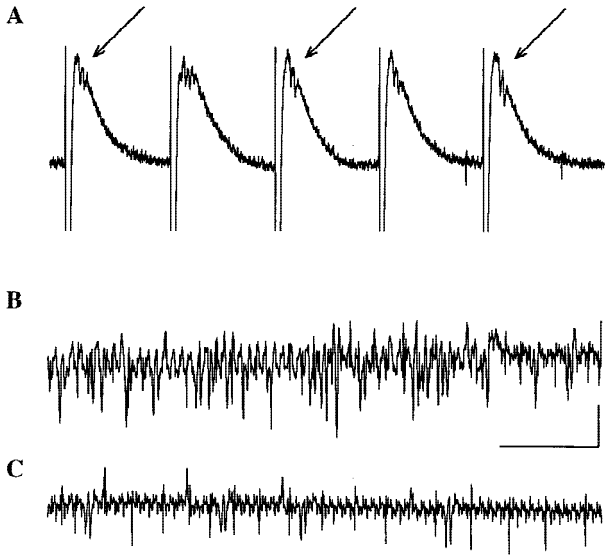

FIGURE 3. Myographic recordings. **(A)** A myographic recording from the exterior dorsal side of the crayfish abdomen at the wire placements indicated in Figure 1. The recording displays the muscle activity during the 2-hour stimulus regime of 5 Hz/s followed by 1 s of rest. The duration of the muscle activity (marked with an arrow) is being followed throughout the conditioning. **(B)** Myographic recordings of the crayfish abdominal muscles during voluntary movement without stimulation. **(C)** Myographic recording of the decrease in activity following voluntary movement as the muscle activity returns to a resting state. Scale bar: **A,** 300 μV; **B, C,** 100 μV; for all 0.2 s.

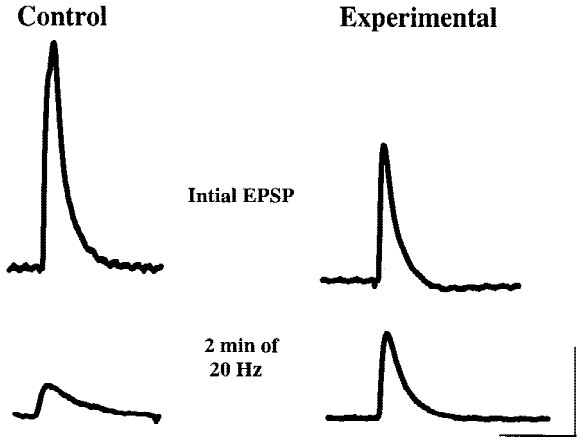

FIGURE 4. The characteristic EPSP in control and conditioned muscles. Top: The initial onset of stimulation. Bottom: At the time the control muscle had fully fatigued. Scale bar: 12 mV, 48 ms.

Physiological Alterations. When phasic motor neurons in crayfish are stimulated at low frequencies (<10 Hz), they exhibit depression or fatigue.[5,7,31,48] In contrast, the tonic motor neurons display facilitation of the synaptic response in response to moderate (10–20 Hz) and high (50–100 Hz) stimulus frequencies.[5,13] In purely phasic muscles and in the phasic response of dually (phasic and tonic) innervated muscles the initial EPSP is larger than for a tonic motor neuron. The initial amplitude of the deep extensor muscles in controls is larger than the conditioned preparations after 1 week. This has been consistently observed in a number of studies[11,12,31,33,34,39,40] showing that after conditioning of a phasic motor neuron the initial EPSP amplitude is smaller. We have also demonstrated this phenomenon after 3 weeks of conditioning (Fig. 4). Further, the conditioned preparation is depression-resistant after continuous stimulation at 20 Hz; the contralateral control muscles within the same segment of the same animal remain unaffected. This type of response is consistently observed after 1, 2, and 3 weeks of conditioning, albeit with substantial animal-to-animal variation (Fig. 5). The responses for two preparations at 1, 2, and 3 weeks are therefore graphed separately. It should be noted that even dur-

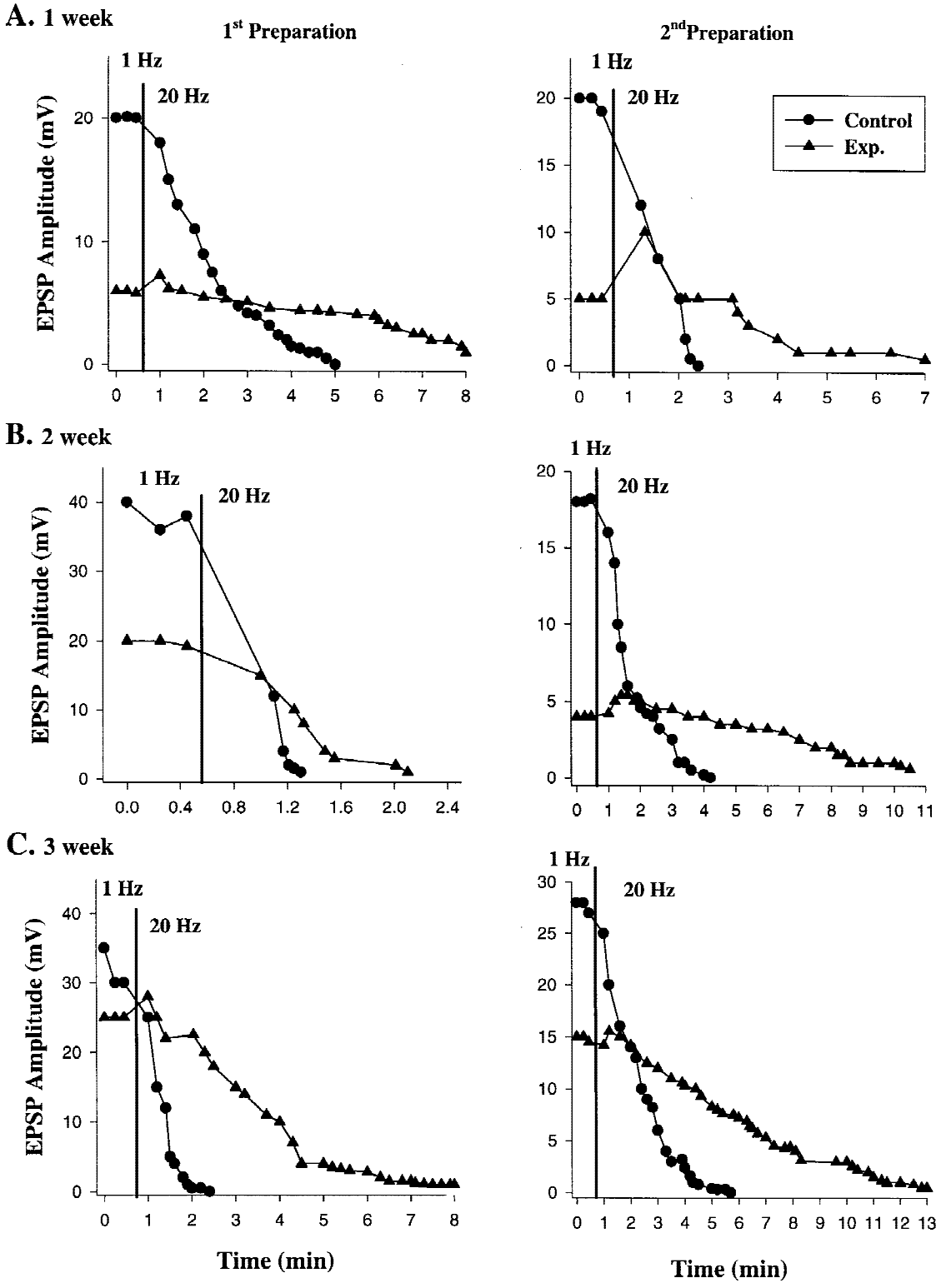

FIGURE 5. Time course of fatigue in EPSPs. **(A)** After 1 week of conditioning the experimental side of a crayfish shows a pronounced fatigue as compared to the control side within a given preparation. **(B)** A second set of crayfish conditioned for 2 weeks. **(C)** A third pair of crayfish conditioned for 3 weeks. The muscles from these sets of crayfish were later processed for protein profiling by SDS gel electrophoresis. Note the difference in time course among preparations but that the trend within individual animals is consistently the same. Stimulation was given at 1 Hz for the first 45 s, then gradually increased to 20 Hz over the next 15 s. After the first minute the stimulation was continuously 20 Hz applied until the preparation showed substantial fatigue. Each point is an average of 10 individual EPSP amplitudes preceding the graphed time point.

ing the 1-Hz period preceding the high-frequency (20 Hz) test for depression, there is a slight trend of depression over the initial 45-s period in the controls which is not evident in the conditioned preparations. During the 20-Hz stimulation period depression is very rapid in the control group as compared to the conditioned muscles, whereas there is a slight facilitation within the first ½ min in five out of the six conditioned preparations followed by a gradual depression over time. This initial facilitation upon high-frequency stimulation was never seen in the control groups. The 20-Hz stimulation is a higher frequency than used in previous studies; but since the frequency within the bursts of voluntary activity is within this range, it is fitting to use this stimulation frequency. Examination of the EPSP duration and decay among the control and conditioned preparations did not reveal any consistent trends (Fig. 4).

Myofibrillar Proteins. The protein banding pattern in 10% SDS–polyacrylamide gels consistently revealed unique profiles for control tonic and phasic muscles. As seen in Figure 6, the two tonic muscles, SLE and SME, have similar patterns. Likewise the phasic L1 and L2 muscles are similar, but distinctly different from the tonic. The protein bands that appear at the top of the gel are myosin heavy chains. The next smaller bands, between 100 and 140 kD, are various paramyosin isoforms, as reported in lobster fast and slow muscles by Mykles.[38] There is a dense, dark-staining band among the isoforms which is associated with the slow (SLE, SME) muscles. The prominent band around 85 kD, pronounced in the fast muscles, has not been reported in the literature. The next lower group of bands has been identified as troponin T isoforms.[38] In lobsters, three different bands were identified among the tonic and phasic muscles, with tonic having an isoform at a slightly larger weight, as shown in Figure 6. The smaller weight isoforms of troponin T run just slightly above the pronounced actin band and are hard to distinguish with Coomassie stain in the presence of large amounts of actin. The band under actin is in both fast and slow fibers and is reported to be tropomyosin.[38] The group of bands in the range of 25–30 kD are various forms of troponin I; lobster muscle contains five such isoforms, two of which are associated with slow muscles.[38] The bands below 20 kD have not been well characterized, but may contain the myosin light chains. The profile differences among the tonic and phasic muscles allow us to identify alterations during the conditioning paradigms.

After 1 week of conditioning no major differences in the SDS gel banding patterns of phasic or tonic muscles from sham (right side) or the conditioned (left side) preparations could be seen (Fig. 7). To obtain better resolution among the high molecular weight proteins, the same protein samples of Figure 7A were run for a longer time to generate the pattern of Figure 7B. But again there was no difference between the sham control and the conditioned muscles. No differences were observed after 2 weeks of conditioning (data not shown), but by 3 weeks of conditioning, difference in the gel banding patterns appeared. The tonic fibers on the sham side did not show any significant changes compared to tonic fibers on the conditioned side nor to tonic fibers after 1 week of conditioning or to unmanipulated controls. The phasic muscles in the conditioned animals (L1, L2, and M) all started to show bands that are normally present only in tonic fibers. The sham group and unoperated control phasic muscles re-

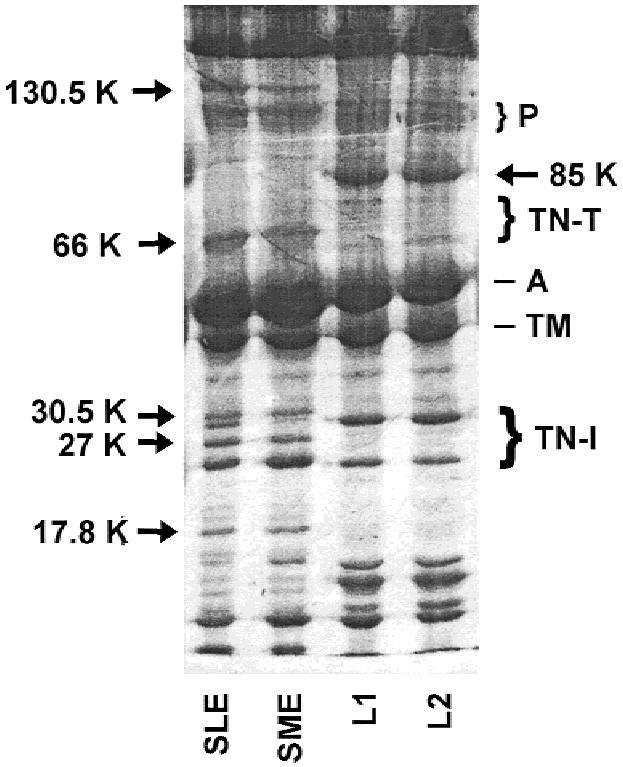

FIGURE 6. Control. A Coomassie-stained SDS–polyacrylamide gel of the tonic and phasic muscle in the second segment of the crayfish abdomen. These samples serve as the control, having had no conditioning or other electrical stimulation. The presence or absence of bands marked with arrows was used to identify transformation in the conditioned muscle samples. The tonic muscles indicated here are the SLE (superficial lateral extensor) and the SME (superficial medial extensor). The phasic muscles include the L1 and L2 (lateral) fibers. The muscle protein variants are also labeled. The variants are used to indicate muscle transformation. P, paramyosin; TN-T, troponin T; A, actin; TM, tropomyosin; TN-I, troponin I; K, kilodaltons.

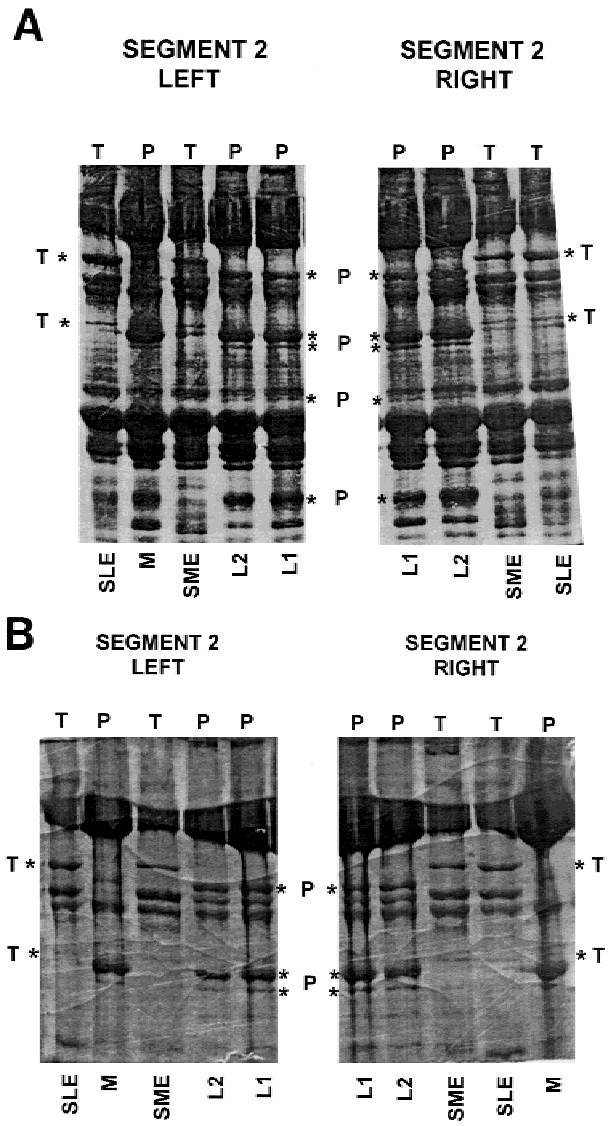

FIGURE 7. One-week of conditioning. **(A)** Protein composition profile of the phasic and tonic muscle from segment 2 of the crayfish abdomen. This samples was taken after 1 week of conditioning the muscle. The left segment is the conditioned sample, and the right is the unstimulated sham control. The muscle tissues were classified as phasic or tonic, and the bands of interest (those indicated in Fig. 1) were marked (*) as characteristic of phasic or tonic. **(B)** Separation among the higher molecular weight bands expanded by 2 hour longer electrophoresis of the sample displayed in Figure 6A. Again the left and right segments are separated, muscle types labeled, and the respective bands marked (*) as either phasic or tonic. There appears to be no transformation in the phasic muscle toward tonic. SLE, superficial lateral extensor; SME, superficial medial extensor; M, medial; L1 and L2, lateral; P, phasic; T, tonic.

tained those bands distinctive of phasic muscles (Fig. 8). All the bands unique to tonic fibers appeared in conditioned muscles, although at levels lower than pure tonic fibers. The altered bands are the paramyosin isoforms, the unidentified 85-kD protein, the troponin T isoforms, troponin I isoforms, and the unidentified lower molecular weight bands. There did not appear to be a diminution of intrinsic phasic proteins. A more refined technical approach to determine the relative ratios of the various proteins would need to be used to make quantitative assessments. We are currently attempting a silver staining method and better protein isolation techniques to resolve the amounts of the various proteins within these muscles under the experimental conditions mentioned in this study.

DISCUSSION

The results of this study extend knowledge of the effects of long-term conditioning of phasic motor neurons innervating phasic muscles in crustaceans. We demonstrate that after 1 week of electrical conditioning the synaptic efficacy of phasic motor neurons is transformed to a more tonic-like state in regard to their evoked responses. After conditioning, the initial EPSP amplitude is reduced and there is an increase in resistance to synaptic fatigue. These transformations are maintained through 3 weeks of

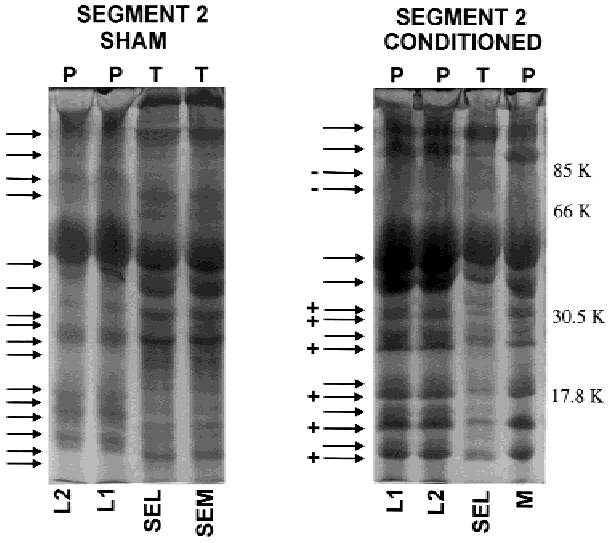

FIGURE 8. Three weeks of conditioning. SDS–polyacrylamide gel electrophoresis analysis of phasic (P) and tonic (T) muscle from segment 2 of the crayfish abdomen after 3 weeks of conditioning. Left: unstimulated sham control (sham), Right: conditioned. Phasic proteins and protein isoforms have decreased, and tonic isoforms have appeared in these phasic muscles, clearly seen at bands of particular interest. SEL, superficial extensor lateral; SEM, superficial extensor medial; M, medial; L1 and L2, lateral; K, kilodaltons. The symbol + indicates it went up in expression, whereas the − sign indicates a decrease among the phasic muscles.

conditioning but are not necessarily increased after the first week. These changes appear to be presynaptic, since no major changes were observed in muscle proteins during the first and second weeks of conditioning. Only after the third week of conditioning was there any sign of altered muscle proteins expression. Previous work on this preparation addressed neither the properties of the muscle fiber types nor whether the muscles showed any degree of conversion to toniclike status. Atwood and Nguyen[5] did assess transformed properties of the motor neuron after 1 week of conditioning in the claw and abdomen, clearly demonstrating that the alterations are due to protein synthesis in the neuron cell body and that these proteins must be transported to the terminal before synaptic efficacy is altered. The results of the present study indicate that only after 3 weeks of conditioning is there an appearance of some degree of protein transformation in the phasic muscles to toniclike profiles; no discernible effects on the muscle proteins could be determined after 1 or 2 weeks of conditioning.

It has been demonstrated in mammalian systems that the characteristics of skeletal muscle fibers are partly due to the activity of the innervating motor neuron.[16,24] Likewise in the crayfish, experimental alteration of nerve impulse activity demonstrates that nerve terminal morphology and metabolic properties are dependent on the electrical activity of the neuron.[31]

Earlier attempts to identify the proteins responsible for the neuronal transformation in this system were made by amplifying and analyzing complementary DNAs derived from the identified common excitatory phasic motor neuron,[7,43] and are still underway awaiting more sequencing information. The advantage of this crustacean preparation over comparable mammalian systems is that only very few, three or four, motor neurons innervate any particular muscle. The motor neurons are identifiable by their terminals on the muscle, and for the most part, their cell bodies are identifiable within the ganglia of the ventral nerve cord. The majority of suitable crustacean muscles are composed of bundles of only 3–5 fibers. Assessment of physiological parameters by intracellular recording within the muscle fibers is well established and straightforward in the crayfish, because such recordings can be done at room temperature, under static or saline perfusion conditions, and because of the simplicity of the saline and its buffering. Regulation of muscle fiber type determination is an intensely investigated area. Of particular interest are the effects of neural influence on muscle fiber type differentiation and the intrinsic nature of muscle in the absence of neural inputs. A recent review by Pette and Staron[44] deals comprehensively with fiber type transitions.

The experimental manipulations described in this study will allow further investigation of plasticity among motor neurons and muscles under controlled laboratory conditions. But it is also of interest to know the degree of plasticity which occurs within the animal in its natural environment and to what extent the ability to alter synaptic efficacy and muscle dynamics serves the animal. Since crayfish do alter their behavior in relation to seasonal variation and the molt cycle, there are relatively long-term activity differences in their neuromuscular systems. It has been shown that the phasic motor nerve terminals of claw closer muscles exhibit the classic phasic morphology during the winter, but swell and become more varicose along the length of the terminal during the summer months.[29,35] The larger terminals, the ones with greater mitochondrial cross-sectional area, are linked to the increase in resistance to synaptic fatigue (i.e., depression). Plasticity in synaptic efficacy in live crustaceans varies also with age and type of neuromuscular unit, as well as from species to species.[3] The claw phasic motor neurons of young crayfish show a greater tendency to undergo conditioned phasic to toniclike transformation than do those of mature animals. At the same time, however, the abdominal phasic system has the ability to transform throughout adulthood. Having the capability to transform the abdominal neuromuscular system would allow the animal to modify its escape response to fit changing conditions and thus provide more effective adaptation to different environments and improve species survival. The advantage to becoming fatigue-resistant in an escape reflex can be a strategy for survival when predators are abundant, although it may cost the animal more energy to maintain such a status. In the situation where predation decreases, the animal can conserve energy by reverting to a phasic, lower-energy state of neuromuscular maintenance.

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This article is dedicated to Dr. H.L. Atwood in his 60th year for

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