ELSEVIER

Comparative Biochemistry and Physiology Part A 127 (2000) 495–504

CBP

www.elsevier.com/locate/cbpa

Sensitivity of transformed (phasic to tonic) motor neurons to the neuromodulator 5-HT

B. Griffis, P. Bonner, R.L. Cooper *

Thomas Hunt Morgan School of Biological Sciences, University of Kentucky, 100 T.H. Morgan Building, Lexington, KY 40506-0225, USA

Received 24 April 2000; received in revised form 1 August 2000; accepted 17 August 2000

Abstract

Long-term adaptation resulting in a 'tonic-like' state can be induced in phasic motor neurons of the crayfish, *Procambarus clarkii*, by daily low-frequency stimulation [Lnenicka, G.A., Atwood, H.L., 1985b. Long-term facilitation and long-term adaptation at synapses of a crayfish phasic motoneuron. J. Neurobiol. 16, 97–110]. To test the hypothesis that motor neurons undergoing adaptation show increased responses to the neuromodulator serotonin (5-HT), phasic motor neurons innervating the deep abdominal extensor muscles of crayfish were stimulated at 2.5 Hz, 2 h/day, for 7 days. One day after cessation of conditioning, contralateral control and conditioned motor neurons of the same segment were stimulated at 1 Hz and the induced excitatory post-synaptic potentials (EPSPs) were recorded from DEL_1 muscle fibers innervated by each motor neuron type. Recordings were made in saline without and with 100 nM 5-HT. EPSP amplitudes were increased by 5-HT exposure in all cases. Conditioned muscles exposed to 5-HT showed a 2-fold higher percentage of increase in EPSP amplitude than did control muscles. Thus, the conditioned motor neurons behaved like intrinsically tonic motoneurons in their response to 5-HT. While these results show that long-term adaptation (LTA) extends to 5-HT neuromodulation, no phenotype switch could be detected in the postsynaptic muscle. Protein isoform profiles, including the myosin heavy chains, do not change after 1 week of conditioning their innervating motor neurons.
© 2000 Elsevier Science Inc. All rights reserved.

Keywords: Phasic; Tonic; Muscle; Motor neuron; Crayfish; Neuromodulator; 5-HT

1. Introduction

In the crayfish, *Procambarus clarkii*, phasic motor neurons normally transform to a more tonic phenotype during seasonal behavioral changes (Lnenicka, 1993; Lnenicka and Zhao, 1991). Transformed motor neurons exhibit decreased initial output and increased varicosity size. Size and number of mitochondria are also increased (Lnenicka, 1993; Lnenicka and Zhao, 1991). Similar motor neuron phenotype changes of varicosity size, mitochondria, and synaptic ultrastructure can be induced experimentally (Lnenicka and Atwood, 1985a,b, 1988, 1989; Lnenicka et al., 1986, 1991). Such adapted motor neurons also demonstrate lower initial output and higher fatigue resistance than do phasic motor neurons. These phenotypic alterations are the phenomenon of long-term adaptation (LTA) (Lnenicka and Atwood, 1985b). Phasic-to-tonic modulation of the type demonstrated in crustaceans appears to occur in many other organisms, including verte-

* Corresponding author. Tel.: +1-606-2571717; fax: +1-606-2575950.

E-mail address: rlcoop1@pop.uky.edu (R.L. Cooper).

1095-6433/00/$ - see front matter © 2000 Elsevier Science Inc. All rights reserved.
PII: S1095-6433(00)00270-1

brates (Robbins, 1980; Sterz et al., 1983) and insects (Irving and Miller, 1980; Titmus, 1981; Rheuben, 1985). LTA represents a major investment by organisms to alter their motor neuron synaptic transmission.

Plasticity of motor neurons is inducible as well by neuromodulators such as serotonin (5-HT). The presence of serotonin and other biogenic amine-containing nerve terminals in the peripheral nervous systems of invertebrates suggests that the amines may not be directed simply at discrete postsynaptic targets. Rather these compounds may be released more diffusely and play a broad neuromodulatory role, even at neuromuscular junctions (Beltz and Kravitz, 1982). Release over a wide area can allow interaction with receptors of several different target cells, which are able to simultaneously respond to transmitters different from the modulator. Depending on the physiological state of the animal, the modulators may be released acutely, as in the case of a transient environmental stimulus, or chronically, for instance when the animal is in a social interaction to establish a hierarchical structure (Huber and Delago, 1998; Huber et al., 1997a,b; Listerman et al., 2000; Sneddon et al., 2000). These sorts of neuromodulation are implicated in relatively slow, long-lasting alterations of the efficacy of synaptic transmission (Kupfermann, 1979). Specific studies addressing the effects of 5-HT and other neuromodulators (octopamine, proctolin, FMRFamides, dopamine) have revealed that they all act on muscle to increase contraction (Dudel, 1965; Kravitz et al., 1980; Mercier and Wilkens, 1985). Furthermore, 5-HT applied to crustacean motor nerve terminals increases transmitter release (Dudel, 1965; Glusman and Kravitz, 1982; Crider and Cooper, 1999; Southard et al., 2000).

Both LTA and the seasonally induced switch in phenotype are apparently the result of increased locomotor activity of the animal. The motor neurons fire more often and, therefore, adapt to be able to resist fatigue, and idea supported by the interesting observation that exercise increases the levels of 5-HT in the hemolymph of shore crabs (Sneddon et al., 2000). Possibly this rise in 5-HT helps resist the synaptic fatigue induced by exercise.

Since LTA and serotonin levels are related to increased locomotor activity in crustaceans, we address the relationship between 5-HT and motor neurons that have undergone LTA. Preliminary work from this lab (Shearer and Cooper, 1999) suggested that tonic nerve terminals exposed to 5-HT produce a greater enhancement of EPSP amplitude than do phasic nerve terminals. Here, we examine the hypothesis that phenotype transformation extends as well to the responsiveness of terminals to 5-HT. Therefore, we postulate that phasic terminals, when transformed to a tonic-like state, will be more responsive to 5-HT than are non-transformed phasic terminals, and will exhibit behavior similar to intrinsically tonic terminals. We extend analysis of major contractile proteins after LTA (Cooper et al., 1998) to myosin heavy chain since this protein is the major regulator of contractile speed and has not been examined previously. Portions of this work have appeared in abstract form (Griffis et al., 1999).

2. Methods

2.1. Animals

Intermolt crayfish (*P. clarkii*, Atchafalaya Biological Supply Co., Raceland, LA) measuring 6–10 cm in body length were used in all experiments. Animals were housed as individuals in an aquatic facility and fed fish food.

2.2. Conditioning

The segmental nerve and associated muscles of the crayfish abdomen are arranged in a fashion that is predictable based on external demarcations of the cuticle (Pilgrim and Wiersma, 1963; Parnas and Atwood, 1966). This allows accurate placement of wire electrodes into selected locations in the animals as previously described (Mercier and Atwood, 1989; Cooper et al., 1998). One set of electrodes straddled the segmental nerve innervating the extensor musculature (Fig. 1, Mercier and Atwood, 1989; Cooper et al., 1998). The second set was placed in the region dorsal to the DEL and SEM muscles on each side of the animal but within the same segment. This is a slightly different arrangement than one reported previously (Cooper et al., 1998). This arrangement was repeated on the contralateral side to provide a control for any effects of electrode placement (i.e. a sham) within the same segment. Care was taken to make sure that after electrode implantation the crayfish had unrestricted movement of the third segment.

Trains of five stimulus pulses were applied through the electrode pair straddling the segmental nerve at 5 Hz every other second (2.5 Hz average impulse frequency), 2 h each day, for 7 consecutive days, as described previously (Mercier and Atwood, 1989; Cooper et al., 1998). Stimulation was confirmed by recording electromyographic (EMG) activity in-phase with all five stimuli through the dorsal electrode pair (Cooper et al., 1998). To maintain conditioning after initial fatigue, minor voltage increases were made as necessary. During conditioning the crayfish, abdomens were flexed. To maintain moisture in the gill chamber and reduce movements that might impair stimulation, the crayfish were placed in moist paper towels and immobilized with latex bands.

2.3. Intracellular recordings

To record intracellularly from DEL_1 muscles, the crayfish were dissected as described previously (Mercier and Atwood, 1989; Cooper et al., 1998) and shown in Fig. 1C. The preparation was rinsed several times with cold crayfish saline before be-

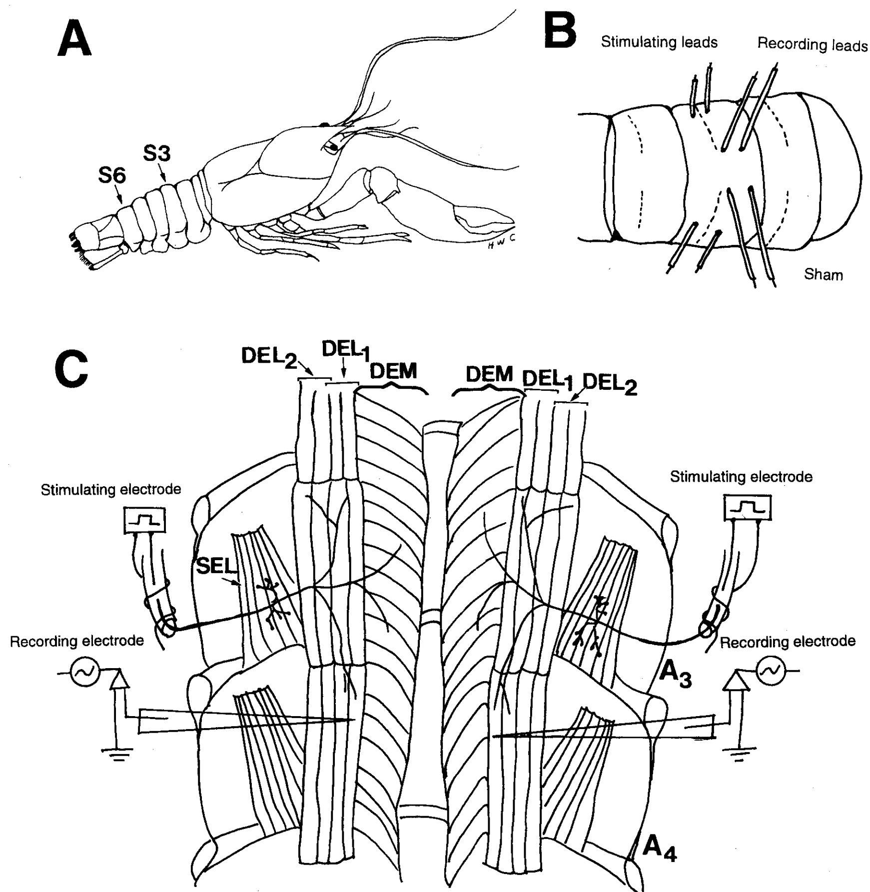

Fig. 1. The placement of the electrodes for long-term conditioning is based on demarcations in the cuticle and their relation to the underlying muscle arrangement. (A) Top view of a crayfish. The electrodes are implanted within abdominal segment 3. (B) An enlargement of abdominal segments 1 through 4 as seen during conditioning. The flexed abdomen helps provide a stable positioning of the nerve to ensure stimulation as well as aiding in keeping the animal still. The stimulating wires are placed so that the nerve root lies between them. 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. (C) A generalized layout showing the deep extensor muscles for segments A3 and A4 and the pattern of innervation to them. The deep extensor lateral 1 (DEL_1), deep extensor lateral 2 (DEL_2), and deep extensor medial (DEM) muscles are all normally fast phenotype muscles. The superficial extensor lateral muscle (SEL) is a slow phenotype muscle. The illustration was based upon photographic montages of methylene blue stained preparations (Sohn et al., 2000). The arrangements for stimulating the segmental nerves within A3 and recording responses from DEL1 muscles in A4 are illustrated.

ginning electrophysiological recording in fresh saline. To determine the effect of 5-HT on the control and conditioned nerves from each animal, intracellular recordings were taken from DEL_1 muscle fibers of the fourth abdominal segment. These fibers are innervated in part by a descending branch of the motor neuron that innervates the DEL_1 muscle of the third segment (Parnas and Atwood, 1966; Lnenicka and Atwood, 1989; Mercier and Atwood, 1989; Sohn et al., 2000). This allowed recordings from fibers innervated by a single identified motor neuron without the possibility that the fibers had been damaged during wire electrode implantation. The nerve roots were stimulated at 1 Hz (0.3 ms pulse duration), with a voltage sufficient to evoke action potentials in the motor neurons, for 5–10 min to establish a baseline excitatory post-synaptic potential (EPSP) amplitude. The saline was then exchanged for saline containing 100-nM 5-HT, and recording continued until an EPSP plateau was reached. Once the EPSPs leveled off, the nerves were stimulated at 5 Hz to test fatigue resistance and confirm the induction of LTA. At fatigue, stimulation was paused for 2 min before again stimulating at 5 Hz. This allowed a measure of recovery, which is also an indicator of LTA.

All EPSP values reported represent the means of at least 29 individual responses. Absolute and percent change in response for the control and conditioned sides of each preparation are presented. The percent difference in increase between the two sides was calculated by the formula [(stimulated − control/control) × 100]. Values were compared using the Wilcoxon non-parametric test.

2.4. SDS-polyacrylamide gel electrophoresis

After electrophysiological recordings were obtained, the DEL_1 muscles were removed and cut in half at their midpoint. Half of each muscle was used for analysis of total muscle protein and one was used for analysis of myosin heavy chain isoforms.

Total muscle protein was purified and separated as previously described (Cooper et al., 1998). One exception was that the gels were run using the Mini-Protean II system (Bio-Rad, Hercules, CA) at 15 mA constant current, until the dye front reached the separating gel, and then at 20 mA until the dye front reached the bottom of the gel. Total run time was about 2 h.

Muscle pieces for myosin extraction were frozen and kept at −80°C until the extraction procedure. Myosin heavy chain extraction and electrophoretic procedures were performed essentially as previously described (LaFramboise et al., 1990, 2000). Gels were pre-stained with Coomassie Blue R-250 in 45% methanol/10% glacial acetic acid, destained in 45% methanol/10% acetic acid, and silver stained according to the Wray procedure (Wray et al., 1981). To improve resolution, gels were washed in 50% methanol overnight before silver staining.

3. Results

The muscles examined in this study were the paired, deep extensor muscles DEL_1 (Fig. 1C) of the dorsal aspect of the crayfish abdomen (Fig. 1A). Implantation of electrodes to stimulate the segmental nerve root as it traverses from the ventral nerve cord to the extensor muscles is simple since indentations of the cuticle (Fig. 1B) show the locations of the SEL muscle attachments and thus the nerve as well. The segmental nerve root can be selectively stimulated when two wires are placed on either side of the cuticular indentations, and the nerve, to a depth of only 1–2 mm under the cuticle (Fig. 1B). Confirmation that the nerve had been stimulated was provided by EMG responses from the corresponding DEM muscles monitored with recording electrodes placed through the dorsal aspect of the abdomen (Fig. 1B). The symmetrical arrangement of muscles within the crayfish abdomen allows the conditioning and control manipulations to be performed on the same animals.

After 1 week of segmental nerve conditioning, the abdomen was surgically removed, the dorsal half of the abdomen exposed (Fig. 1C), and the A3 segmental nerves placed into suction electrodes. The nerves were stimulated through the suction electrodes and the postsynaptic responses of the DEL_1 muscle in A4 were recorded through an intracellular electrode (Fig. 1C). Because the DEL_1 muscle in segment 4 receives motoneuronal innervation from segment 3, this arrangement ensured that responses to only one excitatory motor neuron of the segmental nerve were being monitored (Parnas and Atwood, 1966; Mercier and Atwood, 1989).

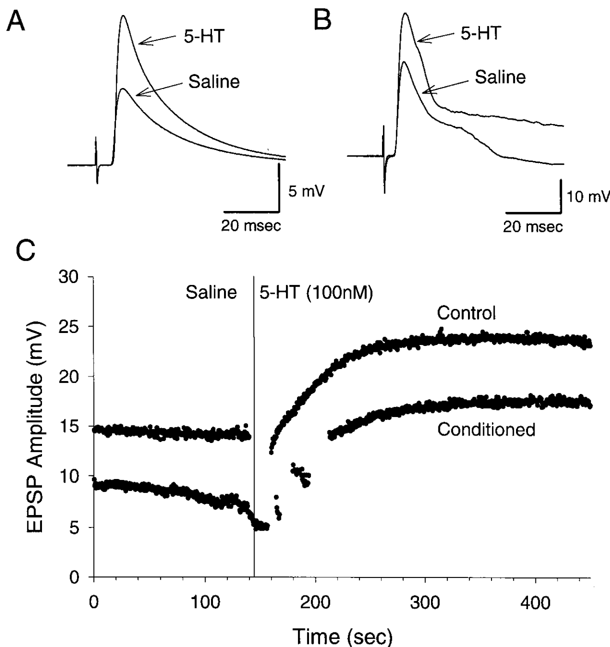

Fig. 2. Characteristic EPSPs from muscles innervated by conditioned (A) and control (B) neurons before and after exposure to 100 nM 5-HT. Note the typically larger initial amplitude from the control. In addition, the larger EPSP events within control muscles sometimes produce twitching which results in artifactual electrical responses after the EPSP (shoulder after the response). (C) A compilation of response measured over the course of one experiment. Notice the rapid rise in EPSP amplitudes after exposure to 5-HT (100 nM). Average EPSP amplitudes measurements can be made from initial baseline and plateau segments (post-5-HT) of the experiment.

The initial EPSP responses are different in amplitude on the conditioned and control sides (Fig. 2A and B). The conditioned muscles typically show smaller initial EPSP amplitudes (Fig. 2A, note scale bar difference). EPSP amplitude from both control and conditioned sides increased after exposure to 5-HT but with larger percentage increases on the conditioned sides (Fig. 2B). The responses throughout one experiment are shown in Fig. 2C. The rapid effect on EPSP amplitude of switching the bathing medium to one containing 100-nM 5-HT is shown as are the pre-treatment baseline and post-treatment plateau regions used to average EPSP amplitudes during each treatment (Fig. 2C). Initial EPSP amplitudes are also varied among preparations. Therefore, for quantitation purposes, the percent change in EPSP amplitude after 5-HT application was calculated (Table 1) along with the absolute change in mV (Fig. 3). In all five preparations, the percent increase in amplitude was larger for the conditioned preparations than the controls ($P < 0.05$, Wilcoxon rank sum test, non-parametric).

Examination of the data also revealed that the absolute change in the EPSP amplitude of the conditioned preparations showed a much smaller degree of variation compared with controls (Table 1). The animal-to-animal variation of mean amplitude, which is typically larger in crayfish preparations compared with vertebrate systems, is greatly decreased by LTA. Although, the mean increase in amplitude is essentially the same for control and conditioned sides, the S.E. decreased from 2.1 in controls to 0.5 in conditioned preparations (Table 1). The raw data of the EPSP amplitudes and their alterations due to exposure of 5-HT (100 nM) are presented in Table 1.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of deep abdominal extensor muscle proteins showed no isoform changes in total muscle protein after 1 week of conditioning, a confirmation of results reported previously (Cooper et al., 1998) nor did myosin heavy chain isoforms change (data not shown). In the present work, we used an improved staining technique (Fig. 4) that more clearly demonstrated not only this lack of total protein change during conditioning but also that DEL_1 retains a clear fast phenotype as evidenced by the presence of the fast muscle-specific protein, P75 (Mykles, 1985a,b; Cooper et al., 1998; Sohn et al., 2000).

4. Discussion

The results of this study support earlier findings (Lnenicka and Atwood, 1985b; Mercier and Atwood, 1989; Lnenicka, 1991; Cooper et al., 1998) that crustacean phasic motor neurons innervating fast-phenotype muscles transform to a tonic-like state within 1 week of activity-conditioning. In keeping with phasic-to-tonic-like conversion, the transformed nerve exhibits greater response to the neuromodulator, 5-HT, than do control-phasic terminals of the same animal. 5-HT enhanced change in EPSP amplitude is proportionally greater for transformed phasic-to-tonic-like nerve terminals, in agreement with the general finding that purely tonic motor neurons are more responsive than phasic motor neurons to 5-HT enhancement of postsynaptic responses (Shearer and Cooper,

Table 1
Comparison of EPSP amplitude before and after 5-HT application

Prep	Side	Pre 5-HT	Post 5-HT	Change	% Change	% Diff.
1	Control	14.2	23.8	9.6	67.6	
	Stim	8.6	17.5	8.9	103.5	53.1
2	Control	15.8	18.9	3.1	19.6	
	Stim	10.4	17	6.6	63.5	224
3	Control	31.3	46.8	15.5	49.5	
	Stim	7.2	15.9	8.7	120.8	144
4	Control	28.5	36.1	7.6	26.7	
	Stim	19.8	27	7.2	36.4	36.3
5	Control	18.1	23.2	5.2	28.2	
	Stim	23.1	31.9	8.8	38.1	35.1
Mean (S.E.M.)						
	Control	21.6 (3.5)	29.8 (5.1)	8.18 (2.1)	38.3 (8.9)	98.5 (37.3)
	Stim	13.8 (3.2)	21.9 (3.2)	8.04 (0.5)	72.5 (17.1)	

1999). While synaptic response parameters show phenotypic transformation after 1 week of activity-conditioning, we have been unable to demonstrate phenotype alteration of protein isoforms, including the myosin heavy chains, purified from muscle fibers innervated by transformed nerves.

Previous investigations of nerve terminal transformation in crayfish showed that some characteristics of transformation, reduction in EPSP amplitude and increase in fatigue resistance, are evident as early as 3 days after beginning electrical conditioning in the phasic motor neuron of the claw closer (Lnenicka and Atwood, 1989). The molecular mechanisms underlying nerve terminal transformation are unclear but appear to depend on the synthesis and transport of proteins within the neurons (Nguyen and Atwood, 1990a,b; Atwood and Cooper, 1996). Ultrastructural changes occurring in transformed nerve terminals include a dramatic increase in varicosity size and greater total cross-sectional area of terminal mitochondria (Lnenicka et al., 1986). Physiological alterations of transformed terminals are reflected by reduced initial EPSPs and, upon repetitive stimulation, development of fatigue resistance (Mercier and Atwood, 1989; Bradacs et al., 1990, 1991).

Because these changes indicate phenotypic conversion to a tonic-like state, we thought it likely that the transformed nerve terminals may also demonstrate another aspect of the tonic state altered responsiveness to neuromodulation. We chose 5-HT, a potent excitatory neuromodulator at crayfish NMJs (Florey and Rathmayer, 1978; Fischer and Florey, 1983; Goy et al., 1984; Southard et al., 2000) to probe this possibility.

5-HT acts via inositol triphosphate (IP_3) and cyclic AMP (cAMP) second-messenger systems (Dixon and Atwood, 1989a,b; Delaney et al., 1991; Braha et al., 1993). At present, it is not known exactly how these second messenger systems enhance synaptic efficacy, but it has been established that 5-HT acts directly to increase the probability of quantal transmitter release (Cooper et al., 1995b; Crider and Cooper, 1999; He et al., 1999; Southard et al., 2000).

Recent studies of high- and low-output terminals on the opener and extensor muscles of crayfish walking legs have demonstrated that the low-output terminals show greater enhancement

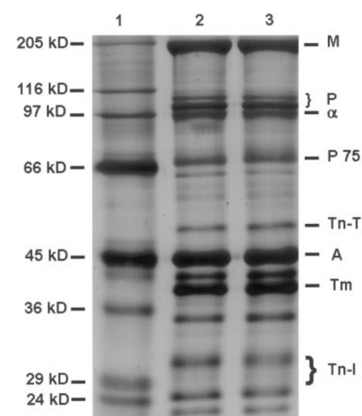

Fig. 3. The absolute change in the EPSP amplitude during exposure to 5-HT varied among preparations. The last set of bars represents the mean (± S.E.M.) for the five preparations. Note that conditioning for 1 week greatly reduced the interanimal variation without changing the mean increase in amplitude. The percent increase in amplitude was always greater for conditioned preparations (Table 1).

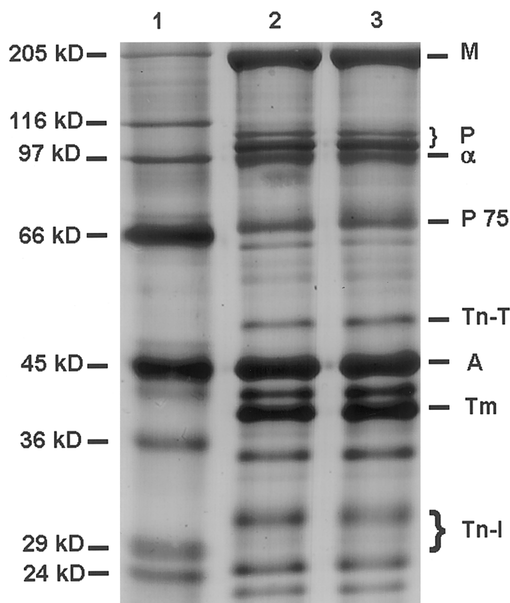

Fig. 4. Electrophoretic separation of muscle proteins after silver staining. Protein profiles from total muscle fractions after separation with 10% SDS-PAGE gels. DEL_1 muscles from control and conditioned sides of the animal are identical in profile. Both show the primary marker of fast pheotype in crustaceans, P75. Several muscle protein variants that can be used to distinguish between fast and slow phenotypes are also labeled and all represent fast isoforms. Lane 1, molecular weight standards (Sigma), 205 kDa (myosin), 116 kDa (B-galactosidase), 97 kDa (Phosphorylase B), 66 kDa (Bovine albumin), 45 kDa (ovalbumin), 36 kDa (G-3-P dehydrogenase), 29 kDa (carbonic anhydrase), 24 kDa (trypsinogen); lane 2, control; lane 3, conditioned. The labeled bands are: M (myosin), P (paramyosin), α (α-actinin), P-75, TN-T (troponin-T), Tm (tropomyosin), A (actin), TN-I (troponin-I). Note that the myosin isoforms do not separate on this gel.

of synaptic strength following exogenous application of 5-HT (Crider and Cooper, 1999, 2000; Shearer and Cooper, 1999). The greater change produced by 5-HT in terminals that started with smaller EPSP amplitudes, as compared with larger-EPSP amplitude terminals, cannot be rationalized by the decreased driving gradient at the higher-EPSP terminals. For example, proximal opener muscle fibers with high-output terminals produce EPSPs of about 1 mV, increasing to 2 mV, or more, in the presence of 5-HT. But central fibers of the same muscle, which have low-output terminals, frequently elicit EPSP amplitudes less than 0.5 mV whose proportional increase with 5-HT is higher than that of the high-output terminals (Crider and Cooper, 1999). Since, the resting membrane potentials for these fibers are in the range of -60 to -70 mV, the EPSP amplitudes are still very far removed from the reversal potential and thus the plateau in the EPSPs of the higher-output terminals (Fig. 2) in the presence of 5-HT is not due to the lack of sufficient electrical or chemical driving gradients.

Some, possibly much, of the difference between opener muscle high- and low-output terminal responsiveness to 5-HT may lie with the terminal ultrastructure, synaptic physiology, and calcium response differences (Cooper et al., 1995a,c, 1996a,b). Synapses within terminals in proximal regions of the opener muscle are more complex than in distal regions having multiple active zones per synapse and higher calcium influx rates upon depolarization. The release machinery in such presynaptic nerve terminals could possibly become saturated upon addition of exogenous 5-HT and thus not enhance release to the same degree as can low-output terminals.

There are behavioral and ecological perspectives to this study. During summer months, crayfish in wild populations undergo natural phasic motor nerve terminal transformation to a more tonic-like, fatigue resistant state (Lnenicka and Zhao, 1991; Lnenicka et al., 1991; Lnenicka, 1993). Our work suggests that these naturally transformed NMJs may also be more responsive to neuromodulation by 5-HT. This correlates with the fact that it is during the summer months that the animals are more active and are establishing their social structure, a process likely to be under the influence of 5-HT (Huber et al., 1997a,b; Sneddon et al., 2000).

The fact that the mean absolute change after 5-HT exposure was essentially the same for the control and conditioned sides may indicate that 5-HT acts upon the same pool of vesicles in each case. However, the great disparity among control preparations indicates that their response to 5-HT is less well-regulated. This makes sense if one considers the compensatory effects of 5-HT on low-output terminals by enhancing the EPSP amplitude. Since the high-output terminals are already generating large changes on the muscle, including induction of contraction without modulation via 5-HT or facilitation, one would not expect their responses to 5-HT to greatly benefit the animal. However, for the low-output terminals, the effects of 5-HT may accelerate the effects of facilitatory trains to generate muscle responses. Therefore, during the establishment of the social hierarchy an animal could more easily match the

need for fatigue resistance with the need for episodic brief increases in output by making tonic-like terminals that generate predictable responses to 5-HT.

Because of this matching at the neuronal level, muscle phenotype changes may be avoided or delayed. Vertebrate muscle changes occur rapidly (Pette and Vrbová, 1992, 1999) and, to the extent that crustacean and vertebrate systems are similar, we, therefore, would have expected to see muscle changes after 1 week if such neuronal integration effects were not taking place directly on the crayfish muscle. We would still expect crayfish muscles to eventually change, and indeed, this has been shown to occur (Costello and Govind, 1984; Quigley and Mellon, 1984; Cooper et al., 1998).

In mammals, activity of motor neurons helps to establish the skeletal muscle phenotype (Burke, 1981; Eisenberg, 1985; Pette and Staron, 1997). Chronic low-frequency stimulation of mammalian fast muscles can cause transformation to a slow phenotype (Pette and Vrbová, 1992, 1999; Delp and Pette, 1994) as can unloading of muscle (Talmadge et al., 1996; Stevens et al., 1999a,b). Crayfish also show some aspects of activity-dependent muscle–phenotype switching:

Prolonged (3 weeks) electrical conditioning can cause tonic-like isoforms of some muscle proteins to appear in phasic muscle fibers (Cooper et al., 1998). But, 1 week of conditioning, as in the present work, is insufficient to drive such changes. The myosin heavy chains were not examined in the previous study (Cooper et al., 1998), but since there are distinct myosin heavy chain protein isoform differences between normal phasic and tonic muscle fibers in crayfish (LaFramboise et al., 2000), we consider that longer conditioning paradigms may well drive phenotype change in these proteins.

Acknowledgements

Funding was provided by NIH-NIMH Behavioral ecology and comparative neurobiology training grant (B.G), NSF grants IBN-9808631 (R.L.C.) and ILI-DUE-9850907 (R.L.C.).

References

Atwood, H.L., Cooper, R.L., 1996. Synaptic diversity and differentiation: crustacean neuromuscular junctions. Invert. Neurosci. 1, 291–307.

Beltz, B.S., Kravitz, E.A., 1982. Mapping of serotonin-like immunoreactivity in the lobster nervous system. J. Neurosci. 3, 585–602.

Bradacs, H., Mercier, A.J., Atwood, H.L., 1990. Long-term adaption in lobster motor neurons and compensation of transmitter release by synergistic inputs. Neurosci. Lett. 108, 110–115.

Bradacs, H., Mercier, A.J., Atwood, H.L., 1991. Activity-dependent adaption of lobster motor neurons and compensation of transmitter release by synergistic inputs. In activity-driven CNS changes in learning and development. Ann. New York Acad. Sci. 627, 378–380.

Braha, O., Edmonds, B., Sacktor, T., Kandel, E.R., Klein, M., 1993. The contributions of protein kinase A and Protein kinase C to the actions of 5-HT on the L-type Ca^{2+} current of sensory neurons in Aplysia. J. Neurosci. 13, 1839–1851.

Burke, R., 1981. Motor units: anatomy, physiology, and functional organization. In: Handbook of Physiology. The Nervous System. Motor Control, vol. 2. American Physiological Society, Bethesda, pp. 345–422.

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

Cooper, R.L., Marin, L., 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., Atwood, H.L., 1995c. Quantal measurement and analysis methods compared for crayfish and *Drosophila* neuromuscular junctions and rat hippocampus. J. Neurosci. Methods 61, 67–79.

Cooper, R.L., Harrington, C., Marin, L., Atwood, H.L., 1996a. Quantal release at visualized terminals of crayfish motor axon: intraterminal and regional differences. J. Comp. Neurol. 374, 1–18.

Cooper, R.L., Winslow, J., Govind, C.K., Atwood, H.L., 1996b. Synaptic structural complexity as a factor enhancing probability of calcium-mediated transmitter release. J. Neurophysiol. 75, 2451–2466.

Cooper, R.L., Warren, W.M., Ashby, H.E., 1998. Activity of phasic motor neurons partially transforms the neuronal and muscle phenotype to a tonic-like state. Muscle Nerve 21, 921–931.

Costello, W.J., Govind, C.K., 1984. Contractile proteins of fast and slow fibers during differentiation of lobster claw muscle. Dev. Biol. 104, 434–440.

Crider, M.E., Cooper, R.L., 1999. Importance of stimulation paradigm in determining facilitation and effects of neuromodulation. Brain Res. 842, 324–331.

Crider, M.E., Cooper, R.L., 2000. Differentially facilitation of high- and low-output nerve terminals from a single motor neuron. J. Appl. Physiol. 88, 987–996.

Delaney, K., Tank, D.W., Zucker, R.S., 1991. Presynaptic calcium and serotonin-mediated enhancement of transmitter release at crayfish neuromuscular junction. J. Neurosci. 11, 2631–2643.

Delp, M.D., Pette, D., 1994. Morphological changes during fiber type transitions in low-frequency-stimulated rat fast-twitch muscle. Cell Tissue Res. 277, 363–371.

Dixon, D., Atwood, H.L., 1989a. Adenyate cyclase system is essential for long-term facilitation at the crayfish neuromuscular junction. J. Neurosci. 9, 4246–4252.

Dixon, D., Atwood, H.L., 1989b. Conjoint action of phosphoinositol and adenylate cyclase systems in serotonin-induced facilitation at the crayfish neuromuscular junction. J. Neurophysiol. 62, 1251–1259.

Dudel, J., 1965. Potential changes in the crayfish motor nerve terminal during repetitive stimulation. Pflug. Arch. 282, 323–337.

Eisenberg, B.R., 1985. Adaptability of ultrastructure in the mammalian muscle. J. exp. Biol. 115, 55–68.

Fischer, L., Florey, E., 1983. Modulation of synaptic transmission and excitation-contraction coupling in the opener muscle of the crayfish, *Astacus leptodactylus*, by 5-hydroxytryptamine and octopamine. J. Exp. Biol. 102, 187–198.

Florey, E., Rathmayer, M., 1978. The effects of octopamine and other amines on the heart and on the neuromuscular transmission in decapod crustaceans: further evidence for a role as a neurohormone. Comp. Biochem. Physiol. 61C, 229–237.

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

Goy, M.F., Schwarz, T.L., Kravitz, E.A., 1984. Serotonin-induced protien phosphorylation in a lobster neuromuscular preparation. J. Neurosci. 4, 611–626.

Griffis, B., Bonner, P.H., Cooper, R.L., 1999. Increased sensitivity of transformed (phasic to tonic-like) motoneurons to the neuromodulator 5-HT. Abst. Soc. Neurosci. 25, 792–813.

He, P., Southard, R.C., Whiteheart, S.W., Cooper, R.L., 1999. Role of α-SNAP in promoting efficient neurotransmission at the crayfish neuromuscular junction. J. Neurophysiol. 82, 3406–3416.

Huber, R., Delago, A., 1998. Serotonin alters decisions to withdraw in fighting crayfish, *Astacus astacus*: the motivational concept revisited. J. Comp. Physiol. 182, 573–583.

Huber, R., Orzeszyna, M., Pokorny, N., Kravitz, E.A., 1997a. Biogenic amines and aggression: experimental approaches in crustaceans. Brain Behav. Evol. 50, 60–68.

Huber, R., Smith, K., Delago, A., Isaksson, K., Kravitz, E.A., 1997b. Serotonin and aggressive motivation in crustaceans: altering the decision to retreat. Proc. Natl. Acd. Sci. USA 94, 5939–5942.

Irving, S.N., Miller, T.A., 1980. Ionic differences in 'fast' and 'slow' neuromuscular transmission in body wall muscles of *Musca domestica* larvae. J. Comp. Physiol. 135, 291–298.

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

Kupfermann, I., 1979. Moduatory actions of neurotransmitters. Ann. Rev. Neurosci. 2, 447–465.

LaFramboise, W.A., Daood, M.J., Guthrie, R.D., Moretti, P., Schiaffino, S., Ontell, M., 1990. Electrophoretic separation and immunological identification of type 2X myosin heavy chain in rat skeletal muscle. Biochim. Biophys. Acta 1035, 109–112.

LaFramboise, W.A., Griffis, B., Bonner, P., Warren, W., Scalise, D., Guthrie, R.D., Cooper, R.L., 2000. Muscle type-specific myosin isoforms in crustacean muscles. J. Exp. Zool. 286, 36–48.

Listerman, L., Deskins, J., Bradacs, H., Cooper, R.L., 2000. Measures of heart rate during social interactions in crayfish and effects of 5-HT. Comp. Biochem. Physiol. A. 125, 251–263.

Lnenicka, G.A., 1991. The role of activity in the development of phasic and tonic synaptic terminals. Ann. New York Acad. Sci. 627, 197–211.

Lnenicka, G.A., 1993. Seasonal differences in motor terminals. Comp. Biochem. Physiol. A 104, 423–429.

Lnenicka, G.A., 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., 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., 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., 1989. Impulse activity of a crayfish motoneuron regulates its neuromuscular synaptic properties. J. Neurophysiol. 61, 91–96.

Lnenicka, G.A., Zhao, Y., 1991. Seasonal differences in the physiology and morphology of crayfish motor terminals. J. Neurobiol. 22, 561–569.

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

Lnenicka, G., Hong, S., Combatti, M., LePage, S., 1991. Activity-dependent development of synaptic varicosities at crayfish motor terminals. J. Neurosci. 11, 1040–1048.

Mercier, A.J., Wilkens, J.L., 1985. Modulatory effects of proctolin on a crab ventilatory muscle. J. Neurobiol. 16, 401–408.

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

Mykles, D.L., 1985a. Multiple variants of myofibrillar proteins in single fibers of lobster claw muscles: evidence for two types of slow fibers in the cutter closer muscle. Biol. Bull. 169, 476–483.

Mykles, D.L., 1985b. Heterogeneity of myofibrillar proteins in lobster fast and slow muscles: variants of troponin, paramyosin, and myosin light chains comprise four distinct protein assemblages. J. Exp. Zool. 234, 23–32.

Nguyen, P.V., Atwood, H.L., 1990a. Axotomy-induced temporal dissociation of long-term adaptive changes at neuromuscular synapses of a crayfish phasic motoneuron. Brain Res. 533, 107–112.

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

Parnas, I., 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.

Pette, D., Vrbová, G., 1992. Adaptation of mammalian skeletal muscle fibers to chronic electrical stimulation. Rev. Physiol. Biochem. Pharmacol. 120, 115–202.

Pette, D., Staron, R.S., 1997. Mammalian skeletal muscle fiber type transitions. Int. Rev. Cytol. 170, 143–223.

Pette, D., Vrbová, G., 1999. What does chronic electrical stimulation teach us about muscle plasticity. Muscle Nerve 22, 666–677.

Pilgrim, R.L.C., Wiersma, C.A.G., 1963. Observations on the skeleton and somatic musculature of the abdomen and thorax of *Procambarus clarkii* (Girard), with notes on the thorax of *Panulirus interruptus* (Randall) and *Astacus*. J. Morphol. 113, 453–587.

Quigley, M.M., Mellon, D.F. Jr, 1984. Changes in myofibrillar gene expression during fiber-type transformation in the claw closer muscles of the snapping shrimp, *Alpheus heterochelis*. Dev. Biol. 106, 262–265.

Rheuben, M., 1985. Quantitative comparison of the structural features of slow and fast neuromuscular junctions in Manduca. J. Neurosci. 5, 1704–1716.

Robbins, N., 1980. Plasticity at the mature neuromuscular junction. Trends Neurosci. 3, 120–122.

Shearer, J., Cooper, R.L., 1999. The differential effects of 5-HT on tonic and phasic motor nerve terminals. Am. Zool. 39, 244A.

Sneddon, L.U., Taylor, A.C., Huntingford, F.A., Watson, D.G., 2000. Agonistic behavior and biogenic amines in shore crabs *Carcinus maenas*. J. Exp. Biol. 203, 537–545.

Sohn, J., Mykles, D.L., Cooper, R.L., 2000. The anatomical, physiological and biochemical characterization of muscles associated with the articulating membrane in the dorsal surface of the crayfish abdomen. J. Exp. Zool., in press.

Southard, R.C., Haggard, J., Crider, M.E., Whiteheart, S.W., Cooper, R.L., 2000. Influence of serotonin on the kinetics of vesicular release. Brain Res. 871, 16–28.

Sterz, R., Pagala, M., Peper, K., 1983. Postjunctional characteristics of the endplates in mammalian fast and slow muscles. Pflüg. Arch. 398, 48–54.

Stevens, L., Gohlsch, B., Mounier, Y., Pette, D., 1999a. Changes in myosin heavy chain mRNA and protein isoforms in single fibers of unloaded rat soleus muscle. FEBS Lett. 463, 15–18.

Stevens, L., Sultan, K.R., Peuker, H., Gohlsch, B., Mounier, Y., Pette, D., 1999b. Time-dependent changes in myosin heavy chain mRNA and protein isoforms in unloaded soleus muscle of rat. Am. J. Physiol. 277, C1044–C1049.

Talmadge, R.J., Roy, R.R., Edgerton, V.R., 1996. Distribution of myosin heavy chain isoforms in non-weight-bearing rat soleus muscle fibers. J. Appl. Physiol. 81, 2540–2546.

Titmus, M.J., 1981. Ultrastructure of identified fast excitatory, slow excitatory and inhibitory neuromuscular junctions in the locust. J. Neurocytol. 10, 363–385.

Wray, W., Boulikas, T., Wray, V.P., Hancock, R., 1981. Silver staining of proteins in polyacrylamide gels. Anal. Biochem. 118, 197–203.