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Differences in synaptic output between excitatory and inhibitory motoneurons in a crayfish muscle

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Abstract. A pair of antagonistic motoneurons, one excitatory and one inhibitory, innervates the distal accessory flexor muscle in the walking limb of the crayfish *Procambarus clarkii*. The number and size of synapses formed by these two axons on the muscle fibers (neuromuscular synapses) and on each other (axo-axonal synapses) were estimated using thin-section electron microscopy. Although profiles of nerve terminals of the two axons occur in roughly equal proportions, the frequency of occurrence of neuromuscular synapses differed markedly: 73% were excitatory and 27% were inhibitory. However, inhibitory synapses were 4–5 times larger than excitatory ones, and consequently, the total contact areas devoted to neuromuscular synapses were similar for both axons. Axo-axonal synapses were predominantly from the inhibitory axon to the excitatory axon (86%), and a few were from the excitatory axon to the inhibitory axon (14%). The role of the inhibitory axo-axonal synapse is presynaptic inhibition, but that of the excitatory axo-axonal synapse is not known. The differences in size of neuromuscular synapses between the two axons may reflect intrinsic determinants of the neuron, while the similarity in total synaptic area may reflect retrograde influences from the muscle for regulating synapse number.

Key words: Axo-axonal synapse – Neuromuscular synapse – Motoneuron – Ultrastructure – *Procambarus clarkii* (Crustacea)

Introduction

The crustacean neuromuscular junction has often served as a model for the vertebrate central nervous system (Katz 1966), because synaptic contacts occur at multiple sites along the muscle membrane, and at each synaptic site, transmitter is released in small amounts and the release is modifiable (Atwood 1976; Atwood and Wojtowicz 1986). Resemblance to the central nervous system is also seen in two other features: crustacean muscle is innervated by both excitatory and inhibitory axons, forming neuromuscular synapses in parallel, and the inhibitory axon makes synaptic contact with the excitatory axon, resulting in presynaptic inhibition. All of these features of synaptic operation are easily studied in the crustacean neuromuscular system, because they are experimentally accessible. An added bonus is the fact that these synapses can be studied for single, identifiable neurons since crustacean muscles are innervated by relatively few motoneurons. For these reasons, crustacean neuromuscular systems have received considerable attention in the study of synaptic physiology and plasticity (Atwood and Wojtowicz 1986; Zucker 1989).

Inhibitory innervation of crustacean limb muscles is via common (serving many muscles) and specific (serving a single muscle) axons (Wiens 1989). Neuromuscular synapses of the specific inhibitory axon in the crayfish opener and shore crab stretcher muscle are matched to those of the excitatory axon in the same muscle fiber (Atwood and Bittner 1971). Thus muscle fibers with small, facilitating excitatory postsynaptic potentials tended also to have small, facilitating inhibitory postsynaptic potentials, and the largest inhibitory potentials were seen in fibers with the largest excitatory potentials. Even the presynaptic inhibition was matched in its rate of facilitation to the excitatory and inhibitory postsynaptic potentials in the same muscle fiber. The close matching of synaptic properties between excitatory and inhibitory axons on individual muscle fibers suggests that the fiber may influence synapse formation (Atwood 1976).

While synaptic properties of excitatory and inhibitory axons are similar in crustacean muscle fibers, do these axons, which have antagonistic actions, provide similar degrees of innervation? For instance, are there similar numbers of synapses formed by the excitatory and inhibitory axons in a crustacean muscle? In the crayfish open-

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er muscle, excitatory and inhibitory neuromuscular synapses were similar in size but occurred in a 2:1 ratio in small (1–3-μm) lengths of serially sectioned nerve terminals (Jahromi and Atwood 1974). Inhibitory synapses were also fewer in number than excitatory ones in estimates made on single muscle fibers in the crayfish opener muscle (Florey and Cahill 1982). A complicating factor in the interpretation of results in both studies was the discovery by Wiens (1985) that the crayfish opener muscle receives a branch of the common inhibitory axon in the most proximal region of the muscle, thereby providing triple innervation to this region. Since the location of the fibers in the crayfish opener muscle was not specified in studies estimating relative numbers of excitatory and inhibitory synapses (Jahromi and Atwood 1974; Florey and Cahill 1982), identification of the inhibitory synapses as belonging to the specific inhibitory axon and not the common inhibitory axon must remain tentative.

A more favorable preparation for comparing the frequency of occurrence of antagonistic synapses is the distal accessory flexor muscle in the thoracic limbs of crayfish and lobsters, as it receives a single excitatory axon and a single inhibitory axon (Govind and Wiens 1985); the latter is a branch of the common inhibitory axon that innervates all of the limb muscles (Wiens 1989). Using electron microscopy, which allows us to distinguish between excitatory and inhibitory nerve terminals, we show that the frequency of occurrence of neuromuscular synapses is several fold greater for the excitatory axon compared to the inhibitory axon. However, because inhibitory synapses are significantly larger than excitatory ones, the cumulative synaptic contact area is similar for the two axons.

Another reason for choosing to study the crayfish distal accessory flexor muscle is that the excitatory and inhibitory axons make synaptic contact with each other (axo-axonal synapses) (Pearce and Govind 1993), thereby providing an opportunity not only to examine neuromuscular synapses formed by antagonistic axons onto a common target but also onto each other. We find that the majority of axo-axonal synapses are from the inhibitory axon onto the excitatory axon, while a small minority occur in the reverse direction.

Materials and methods

Adult crayfish (*Procambarus clarkii*) were purchased from the Atchafalaya Biological Supply Company (Raceland, La., USA) and kept in aerated tap water at ambient temperatures. The animals had a carapace length of 4–5 cm and a total body length of 9–10 cm. Only the distal accessory flexor muscle in the first walking leg was used. The animal was made to autotomize the limb and the distal accessory flexor muscle was exposed in the merus segment in cold crayfish saline (Jahromi and Atwood 1974). This was done by chipping away the exoskeleton on the dorsal face and removing the large flexor muscle to expose the underlying distal accessory flexor muscle with minimal dissection in order to preserve its innervation.

The distal accessory flexor muscle was prepared for electron microscopy by published techniques (Govind et al. 1994). Numerous innervation sites were located on the muscle fibers and serially sectioned for various lengths, ranging from 4 to 35 μm. Several sites were chosen at random and extensively photographed for quantitative analysis. A total length of 1200 μm of serially sectioned nerve terminals was examined in this study.

Results

The excitatory and inhibitory axons to the crayfish distal accessory flexor muscle usually travel and branch together on the surface of the muscle fiber, where they differentiate into nerve terminals (Fig. 1). The shape of the synaptic vesicles in these terminals identifies them as excitatory (spherical) or inhibitory (elliptical) (Atwood 1976). The nerve terminal regions are enclosed in large pockets of granular sarcoplasm where intimate contacts are made with the muscle membrane via neuromuscular synapses (Fig. 1A). Additionally, within the confines of the muscle, the excitatory and inhibitory axons also contact each other via axo-axonal synapses (Fig. 1B, C). The frequency of occurrence of these different types of synapses and their size were calculated by examining, with the electron microscope, numerous sites of innervation and randomly selecting a few for quantitative analysis.

Differences between antagonistic axons in neuromuscular synaptic output

In order to characterize the makeup of innervation sites on fibers of the crayfish distal accessory flexor muscle, 83 separate sites were examined from seven muscles obtained from five crayfish. This large sample revealed that most sites possessed both excitatory and inhibitory nerve terminals (Fig. 1), and as a result, the two types of terminals appeared in almost equal proportions in the total count (Table 1). At these innervation sites both types of nerve terminals formed neuromuscular synapses recognizable by the densely stained presynaptic and postsynaptic membranes, separated by a regularly aligned synaptic gap (Fig. 1). The frequency of these synaptic contacts varied markedly between the two axons: most (73%) were excitatory and a few (27%) were inhibitory (Table 1). Thus innervation sites typically contain both axons but the excitatory axon has more synapses than the inhibitory axon.

The asymmetry in the number of synapses between the two axons raises the issue of whether they also differ in size. To obtain information on synaptic size, as well as additional information on their number, five of the 83 innervation sites were serially sectioned (Table 2). At each site excitatory synapses outnumbered inhibitory synapses 2–20-fold. The difference was six-fold over the total number of synapses from all five sites. More importantly for the question at hand, inhibitory synapses at each site were on average five-fold larger than their excitatory counterparts and this was significantly different (Student's *t*-test). We calculated the total synaptic area devoted to each axon type and found the two areas to be similar (Table 2); the excitatory synaptic area was 1.06 times larger than the inhibitory area.

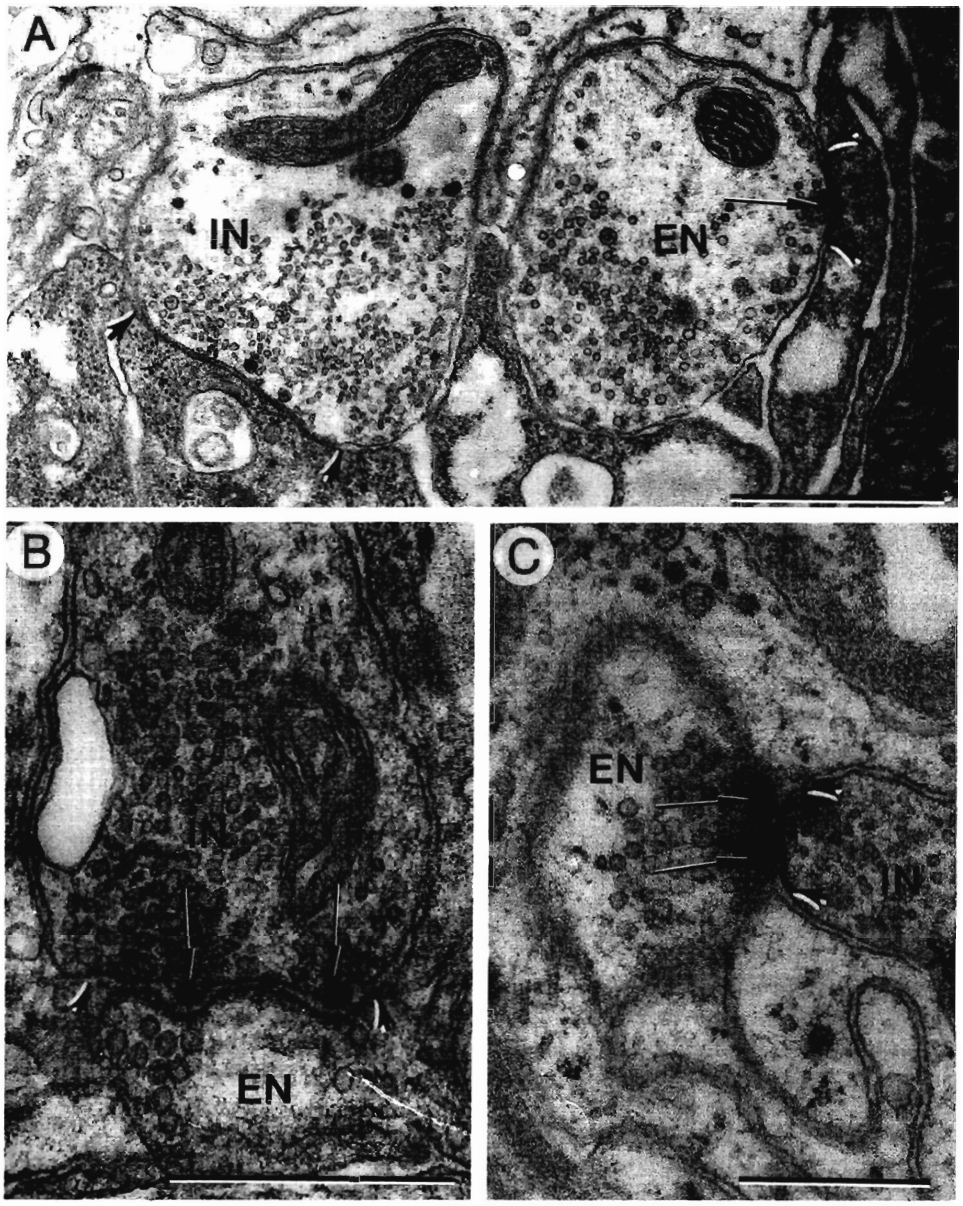

Fig. 1A–C. Fine structure of neuromuscular (**A**) and axo-axonal (**B,C**) synapses in the crayfish distal accessory flexor muscle. Excitatory (*EN*) and inhibitory (*IN*) nerve terminals are identified by the characteristic shape of the clear synaptic vesicles: spherical for excitatory and elliptical for inhibitory. **A** An innervation site showing profiles of excitatory and inhibitory nerve terminals containing mitochondria, many clear synaptic vesicles, and a few dense-core vesicles. Synaptic contacts (between *short arrows*) show densely stained presynaptic and postsynaptic membranes running parallel to each other. A presynaptic dense bar (*long arrow*) with a clustering of synaptic vesicles denotes an active zone at an excitatory synapse. **B** An inhibitory nerve terminal making synaptic contact (between *short arrows*) onto an excitatory nerve terminal. Two well defined presynaptic dense bars (*long arrows*) indicate direction of transmission. **C** An excitatory nerve terminal making synaptic contact (between *short arrows*) onto an inhibitory nerve terminal. Two separate presynaptic dense bars (*long arrows*) on the excitatory membrane indicate direction of transmission. ×28000 (**A**), ×75000 (**B**), ×50000 (**C**). *Bars:* 1 μm (**A**), 0.5 μm (**B,C**)

Table 1. Frequency of occurrence of excitatory and inhibitory nerve terminals and synaptic contacts at sampled innervation sites in the crayfish distal accessory flexor muscle

	Total number	Excitatory nerve terminals		Inhibitory nerve terminals	
		No.	%	No.	%
Innervation sites	83	83	100	69	83
Nerve terminals	291	162	56	129	44
Neuromuscular synapses	435	318	73	117	27
Axo-axonal synapses	21	3	14	18	86

Thus both axons form similar amounts of total synaptic contact area, but the excitatory axon achieves this via many small synapses and the inhibitory axon via fewer, but larger synapses.

Also characteristic of these neuromuscular synapses was the appearance of a presynaptic dense bar with a clustering of synaptic vesicles, denoting an active zone of transmitter release (Fig. 1). Data from the serially sectioned terminals permit comparison of the number and size of these zones (Table 2). At each site the number of dense bars exceeds the number of synapses, because a few synapses possess more than one bar. For instance, 80% of the excitatory synapses in Table 2 showed a single dense bar, and the remaining 20% showed two bars. Amongst the inhibitory synapses, 63% possessed a single dense bar, 28% possessed two bars, and 9% possessed three bars.

Table 2. Size (mean±SD) of synapses and dense bars in concurrent excitatory (E) and inhibitory (I) nerve terminals from five serially sectioned sites of the distal accessory flexor muscles from three crayfish

	E-synapse area (μm^2)	E-dense bar length (μm)	I-synapse area (μm^2)	I-dense bar length (μm)
Site 1	0.153±0.050	0.133±0.055	0.644±0.064	0.103±0.031
(5.2 μm)	n=30	n=34	n=2	n=4
Site 2	0.194±0.089	0.120±0.043	1.794±0.122	0.120±0.035
(5.2 μm)	n=28	n=33	n=3	n=4
Site 3	0.179±0.068	0.122±0.042	0.736±0.045	0.138±0.056
(5.3 μm)	n=13	n=20	n=7	n=12
Site 4	0.156±0.048	0.248±0.099	0.518±0.013	0.224±0.106
(4.3 μm)	n=28	n=33	n=3	n=6
Site 5	0.160±0.039	0.118±0.049	0.623±0.064	0.147±0.056
(4.0 μm)	n=17	n=21	n=7	n=9
All sites	0.167±0.063	0.153±0.082	0.806±0.071	0.149±0.071
(24.0 μm)	n=116	n=141	n=22	n=35
Total size	19.423	21.566	17.735	5.225

Dense bars ranged in length from 0.100 to 0.250 μm with a mean length of about 0.150 μm for both excitatory and inhibitory synapses (Table 2). The means were not significantly different (Student's t-test). However, the total number of dense bars differed between the two axons because of differences in the frequency of synapses. Thus there were four times as many excitatory dense bars as inhibitory ones in the serially sectioned terminals. As a result there was also a four-fold difference in total dense bar length between excitatory and inhibitory axons.

Axo-axonal synaptic output between antagonistic axons

In crustacean muscle the inhibitory axon usually forms the presynaptic component of axo-axonal synapses (Atwood 1976) (Fig. 1B). In the present examination of the crayfish distal accessory flexor muscle, we uncovered 18 such inhibitory axo-axonal synapses, and this constituted the majority (86%) of axo-axonal synapses that we observed (Table 1). However, we also found three cases of the reverse configuration, with the excitatory axon presynaptic to the inhibitory axon (excitatory axo-axonal synapse) (Fig. 1C), in our extensive sampling (Table 1). This number constitutes 14% of the axo-axonal synapses. Clearly, inhibitory axo-axonal synapses occur much more commonly than their excitatory counterparts in the crayfish distal accessory flexor muscle.

The rare occurrence of excitatory axo-axonal synapses precluded measurement of their mean size, although two of the three synapses were serially sectioned and found to have surface areas of 0.285 and 0.927 μm^2. The larger synapse had five dense bars and the smaller one had two; the length of these seven dense bars was 0.157±0.730 μm (mean±SD).

A larger sample size was available for inhibitory axo-axonal synapses and measurements here revealed a size of 0.242±0.126 μm^2 (n=10). Of the ten serially sectioned synapses, three did not show a presynaptic dense bar, five synapses had a single dense bar each, and two synapses had two dense bars each. The mean length of these bars was 0.206±0.138 μm (n=9).

Although data on synaptic size is insufficient for meaningful comparisons, data on frequency of occurrence clearly indicate that the inhibitory axon makes many more axo-axonal synapses than the excitatory axon in the crayfish distal accessory flexor muscle.

Proportion of neuromuscular to axo-axonal synaptic output

The relative frequency of axo-axonal and neuromuscular synapses for each axon can be calculated from Table 1; for the excitatory axon there were 318 neuromuscular and 3 axo-axonal synapses, giving values of 99% and 1%, respectively, and for the inhibitory axon there were 117 neuromuscular and 18 axo-axonal synapses, giving values of 85% and 15%, respectively. Thus the primary target for each axon is the muscle while the secondary target is the antagonistic axon.

Discussion

Differences between antagonistic axons in neuromuscular synaptic output

Our study examines the response of two antagonistic axons to a common developmental target by estimating the frequency and size of neuromuscular synapses. The principal finding is that the excitatory axon forms many small synapses while the inhibitory axon forms fewer, but larger synapses. Since almost all synapses have at least one presynaptic dense bar or active zone and the dense bars are of standard length, the distal accessory flexor muscle receives excitatory innervation via many small functional units and inhibitory innervation via a few large functional units. Such differences may reflect differences in modulation of transmitter release between the two axons. For example, the smaller excitatory synapses may be more fully activated at a low firing frequency while the large inhibitory synapses may require higher firing frequencies for more complete activation. Differences in size of synapses are associated with dif-

ferences in physiological performance between the fast and slow excitatory axons to the claw closer muscles in lobster (Hill and Govind 1981) and crayfish (Lnenicka et al. 1986).

The size differences between excitatory and inhibitory neuromuscular synapses in the crayfish distal accessory flexor muscle is commonplace amongst crustacean muscles; in each case where mean synaptic size has been calculated, the inhibitory synapse is significantly larger than the excitatory one. This is the case for muscles innervated by the common inhibitory axon and a single excitatory axon, such as the proximal (Govind and DeRosa 1983; Govind and Pearce 1989) and distal (Pearce et al. 1985) heads of the accessory flexor muscle and the rotator muscle (Wiens and Govind 1990) in the lobster limb. A similar trend prevails in the case of the shore crab claw closer muscle innervated by a common inhibitory axon and two excitatory axons (Read and Govind 1993) and for the lobster limb flexor (Wiens et al. 1991) and deep abdominal extensor (Govind et al. 1985) muscles innervated by a common inhibitory axon and several excitatory axons. While the foregoing examples are for the common inhibitory axon, synaptic size for specific inhibitory axons has been calculated only for the crayfish opener muscle. Inhibitory synapses were 1.2-fold larger than excitatory ones when synaptic size was calculated from short (1–3-μm) lengths of serially sectioned nerve terminals (Jahromi and Atwood 1974). However, when calculated from much longer (7–22-μm) lengths of nerve terminals so that complete synapses could be measured, the inhibitory synapses were 3–5-fold larger than the excitatory ones (C.K. Govind, C. Gee, and J. Pearce, unpublished observations; Govind et al. 1994). Thus neuromuscular synapses of the common and specific inhibitory axons are consistently larger than their excitatory counterparts. This suggests that synapse size is an intrinsic property of the neuron and unlikely to be influenced to any great extent by the target tissue.

The higher frequency of occurrence of excitatory synapses compared to inhibitory ones in the crayfish distal accessory flexor muscle is also commonplace in crustacean muscle; such a difference has been noted in each of the muscles mentioned above. For example, it is interesting to note that the relative proportion of 73% excitatory and 27% inhibitory synapses in the crayfish distal accessory flexor muscle is closely paralleled in the crayfish opener muscle where values of 64% and 36% were obtained in one sample (Jahromi and Atwood 1974) and 72% and 28% in another (Atwood and Kwan 1976). Moreover, in areas of the crayfish opener muscle innervated by the specific inhibitory axon and not the common inhibitory axon, a sampling of 60 innervation sites revealed a distribution of 66% excitatory and 33% inhibitory synapses (C.K. Govind, C. Gee, and J. Pearce, unpublished observations). Since excitation is a more primary function in muscle, the presence of many small excitatory synapses each with a presynaptic dense bar may not only serve to enhance total transmitter output but also to control its delivery.

Despite differences in size of synapses of the excitatory and inhibitory axons, these antagonistic axons generate similar total amounts of synaptic membrane in the crayfish distal accessory flexor muscle. The consistent nature of these size differences not only in the crayfish distal accessory flexor muscle but in many other crustacean muscles suggests that synapse size may be regulated by the neuron itself. Therefore, regulating the number of synapses formed by each axon may be the only device for generating similar total amounts of synaptic membrane for the antagonistic axons. Such regulation of synapse number may be influenced by the muscle. Postsynaptic regulation of synaptic properties is strikingly demonstrated in an identified sensory neuron in the cricket which forms facilitating synapses on the medial giant interneuron and depressing synapses on interneuron 10–3 (Davis and Murphey 1993).

Differences between antagonistic axons in axo-axonal synaptic output

The usual synaptic contact between motor axons in crustacean muscle is from the inhibitory axon to the excitatory axon (Atwood 1976) and rarely do contacts occur from the excitatory axon to the inhibitory axon. In the crayfish distal accessory flexor muscle, where axo-axonal synapses of both configurations exist, there are few of the putative excitatory output type (16%), while most (84%) are of the inhibitory output type. The relatively higher frequency of occurrence of the inhibitory axo-axonal synapse is in keeping with its role of reducing the effectiveness of the excitatory synapses and thereby providing another means of controlling muscle fiber activation.

On the other hand, the very infrequent occurrence of the putative excitatory axo-axonal synapse makes speculation about its role problematic. The first case of an excitatory axon making synaptic contact with the inhibitory axon was reported in crab stretcher muscle (Atwood and Kwan 1979) which is innervated by common and specific inhibitory axons. More recently this synapse type was found in the crab closer muscle and crayfish distal accessory flexor muscle (Pearce and Govind 1993), both muscles innervated by only a common, not a specific, inhibitory axon. The putative excitatory axo-axonal synapse clearly seems likely to function in the conventional sense as these synapses display several well-defined presynaptic dense bars. Synaptic transmission at these excitatory output synapses would serve to modulate transmitter release at synaptic terminals of the common inhibitory axon, much in the same way that inhibitory axo-axonal synapses modulate transmitter release at synaptic terminals of the excitatory axon. The modulatory effect in the case of the inhibitory output synapse is to reduce transmitter output at excitatory synaptic terminals by suitably timed membrane shunting of the excitatory nerve terminals, i.e., presynaptic inhibition (Atwood 1976). A similar role is possible for the putative excitatory output synapses based on the recent finding of two different species of glutamate and γ-amino butyric acid (GABA) receptors in lobster motor axons (Miwa et al. 1990). In addition to the classical glutamate$_A$ and

$GABA_A$ receptors in the postsynaptic membrane of lobster neuromuscular terminals, a second set, referred to as $glutamate_B$ and $GABA_B$ receptors, was found in the presynaptic membrane of excitatory and inhibitory axons. The newly found $glutamate_B$ and $GABA_B$ receptors both activate K^+ channels via G proteins. Hence glutamate released at the axo-axonal excitatory output synapse would bind to $glutamate_B$ receptors on the inhibitory nerve terminal, resulting in an outward movement of K^+ and a hyperpolarization of the membrane. Such membrane shunting would restrict transmitter output from nerve terminals of the inhibitory axon where excitatory output synapses were located. In other words, putative excitatory axo-axonal synapses may provide presynaptic inhibition of the common inhibitory axon in the crayfish distal accessory flexor muscle.

Another possibility is that these excitatory axo-axonal synapses represent fortuitous contacts because nerve terminals and axon branches of the two axons are closely juxtaposed. Unconventional synaptic contacts between motor axons (Kirk and Govind 1992) or between excitatory axons and glial cells (Govind et al. 1981) have been reported in lobster muscles.

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References

Atwood HL (1976) Organization and synaptic physiology of crustacean neuromuscular systems. Prog Neurobiol 7:291–391

Atwood HL, Bittner GD (1971) Matching of excitatory and inhibitory inputs to crustacean muscle fibers. J Neurophysiol 34:157–170

Atwood HL, Kwan I (1976) Synaptic development in the crayfish opener muscle. J Neurobiol 7:289–312

Atwood HL, Kwan I (1979) Reciprocal axo-axonal synapses between excitatory and inhibitory neurons in crustaceans. Brain Res 174:324–328

Atwood HL, Wojtowicz MJ (1986) Short-term and long-term plasticity and physiological differentiation of crustacean motor synapses. Int Rev Neurobiol 28:275–362

Davis GW, Murphey RK (1993) A role for postsynaptic neurons in determining the presynaptic release properties in the cricket CNS: Evidence for retrograde control of facilitation. J Neurosci 13:3827–3838

Florey E, Cahill MA (1982) The innervation pattern of crustacean skeletal muscle. Cell Tissue Res 224:527–541

Govind CK, DeRosa RA (1983) Fine structure of comparable synapses in a mature and larval lobster muscle. Tissue Cell 15:97–106

Govind CK, Pearce J (1989) Growth of inhibitory innervation in a lobster muscle. J Morphol 199:197–205

Govind CK, Wiens TJ (1985) Innervation of the limb accessory flexor muscle in several decapod crustaceans. I. Anatomy. J Neurobiol 16:317–328

Govind CK, DeRosa R, Pearce J (1981) Presynaptic dense bars at neuromuscular synapses of the lobster, *Homarus americanus*. Cell Tissue Res 207:81–88

Govind CK, Stephens PJ, Eisen JS (1985) Polyneuronal innervation of an adult and embryonic lobster muscle. J Embryol Exp Morphol 87:13–26

Govind CK, Pearce J, Wojtowicz JM, Atwood HL (1994) Strong and weak synaptic differentiation in the crayfish opener muscle: structural correlates. Synapse 16:45–58

Hill RH, Govind CK (1981) Comparison of fast and slow synaptic terminals in lobster muscle. Cell Tissue Res 221:303–310

Jahromi SS, Atwood HL (1974) Three dimensional ultrastructure of the crayfish neuromuscular apparatus. J Cell Biol 63:599–613

Katz B (1966) Nerve, muscle and synapse. McGraw-Hill, New York, p 193

Kirk MD, Govind CK (1992) Early innervation of abdominal swimmeret muscles in developing lobsters. J Exp Zool 261:298–309

Lnenicka GA, Atwood HL, Marin L (1986) Morphological transformation of synaptic terminals of a phasic motoneuron by long-term tonic stimulation. J Neurosci 6:2252–2258

Miwa A, Ui M, Kawai N (1990) G-protein is coupled to presynaptic glutamate and GABA receptors in lobster neuromuscular synapse. J Neurophysiol 63:173–180

Pearce J, Govind CK (1993) Reciprocal axo-axonal synapses between the common inhibitor and excitor motoneurons in crustacean limb muscles. J Neurocytol 22:259–265

Pearce J, Govind CK, Meiss DE (1985) Growth-related features of lobster neuromuscular terminals. Develop Brain Res 21:215–228

Read AT, Govind CK (1993) Fine structure of identified muscle fibers and their neuromuscular synapses in the limb closer muscle of the crab *Eriphia spinifrons*. Cell Tissue Res 271:23–31

Wiens TJ (1985) Triple innervation of the crayfish opener muscle: the astacuran common inhibitor. J Neurobiol 16:183–191

Wiens TJ (1989) Common and specific inhibition in leg muscles of decapods: Sharpened distinctions. J Neurobiol 20:458–469

Wiens TJ, Govind CK (1990) Innervation of the lobster limb rotator muscle: physiology and fine structure. J Exp Zool 253:252–262

Wiens TJ, Pearce J, Govind CK (1991) Neuromuscular properties of the quintuply innervated flexor muscle in lobster limbs. Can J Zool 69:477–488

Zucker RS (1989) Short-term synaptic plasticity. Annu Rev Neurosci 12:13–31