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Loss of Escape-Related Giant Neurons in a Spiny Lobster, *Panulirus argus*

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Abstract. When attacked, many decapod crustaceans perform tailflips, which are triggered by a neural circuit that includes lateral giant interneurons, medial giant interneurons, and fast flexor motor giant neurons (MoGs). Slipper lobsters (Scyllaridae) lack these giant neurons, and it has been hypothesized that behavioral (*e.g.,* digging) and morphological (*e.g.,* flattening and armor) specializations in this group caused the loss of escape-related giant neurons. To test this hypothesis, we examined a species of spiny lobster, *Panulirus argus.* Spiny lobsters belong to the sister taxon of the scyllarids, but they have a more crayfish-like morphology than scyllarids and were predicted to have escape-related giant neurons. Ventral nerve cords of *P. argus* were examined using paraffin-embedded sections and cobalt backfills. We found no escape-related giant neurons and no large axon profiles in the dorsal region of the nerve cord of *P. argus.* Cobalt backfills showed one fewer fast flexor motor neuron than in species with MoGs and none of the fast flexor motor neurons show any of the anatomical specializations of MoGs. This suggests that all palinuran species lack this giant escape circuit, and that the loss of rapid escape behavior preceded, and may have driven, alternative predator avoidance and anti-predator strategies in palinurans.

Introduction

Escape responses are found in many organisms (Eaton, 1984; Otis and Gilly, 1990; Hale *et al.,* 2002; Laura, 2006). The escape response of crayfish and clawed lobsters, which are slow-walking animals, is a powerful, short-latency tailflip (Wine and Krasne, 1972), which significantly increases the probability of surviving a predator's attack (Herberholz *et al.,* 2004). Escape tailflips and their underlying neural bases have been studied extensively in crayfish, particularly *Procambarus clarkii* (Girard, 1852) (Wine and Krasne, 1972, 1982; Wine, 1984; Edwards *et al.,* 1999; Krasne and Edwards, 2002). The crayfish ventral nerve cord contains two bilaterally paired giant axons, the medial giant (MG) and lateral giant (LG), which connect to motor giant (MoG) abdominal flexor neurons. Early research correctly concluded that these giant neurons produce escape tailflips (Johnson, 1926; Wiersma, 1947). Later research showed that although both sets of giant interneurons cause a single rapid abdominal flexion, each set of giant neurons has different triggers and causes a different movement (Wine and Krasne, 1982). The MGs are activated by anterior stimuli, synapse with MoGs in all abdominal segments, and cause the entire abdomen to flex, thereby propelling the crayfish backwards. The LGs are activated by posterior stimuli, synapse with MoGs in the anterior three abdominal segments and thorax, and cause only the anterior portion of the abdomen to flex, thereby causing the posterior end of the crayfish to pitch up (Heitler and Fraser, 1993; Edwards *et al.,* 1999). Giant axons facilitate rapid escape responses because the conduction velocity of an action potential is inversely proportional to axon diameter (Govind and Lang, 1976). Electrical synapses also facilitate rapid escape responses (Furshpan and Potter, 1959). In crayfish, the latency of giant-mediated responses is usually less than 10 ms (Krasne and Wine, 1984; Herberholz *et al.,* 2004). Tailflips can occur without the giant interneurons firing, but such non-giant tailflips have a significantly longer response latency than giant-mediated tailflips (Krasne and Wine, 1984; Herberholz *et al.,* 2004).

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Abbreviations: FAC, flexor anterior contralateral cluster; FMC, flexor medial contralateral cluster; FPI, flexor posterior ipsilateral cluster; LG, lateral giant interneuron; MG, medial giant interneuron; MoG, fast flexor motor giant neuron; $N3_d$, dorsal branch of nerve 3.

Intuitively, short-latency escape responses should provide a clear adaptive advantage to almost any species (Eaton, 1984; Laura, 2006), but little empirical evidence supported this notion until recently (Herberholz *et al.,* 2004; Walker *et al.,* 2005). This putative advantage makes it puzzling that some crustacean species have lost the giant neurons responsible for fast escape responses (Fig. 1). In contrast, fast escape starts in fish are variable across taxa, but there are no documented losses of the primary giant neurons associated with them, the Mauthner cells (Hale *et al.,* 2002). In crustaceans, the loss of the giant escape circuit in the slipper lobster species *Ibacus peronii* Leach, 1815 and *Ibacus alticrenatus* Bate, 1888 (Scyllaridae) is of particular interest here (Faulkes, 2004). These species dig into sand, apparently for predator avoidance (Faulkes, 2004, 2005, 2006), and like other scyllarids, are heavily armored (Barshaw *et al.,* 2003) and capable of powerful and sustained (non-giant) tailflipping (Jacklyn and Ritz, 1986; Jones, 1988; Spanier *et al.,* 1991). Other scyllarids use clinging (Barshaw and Spanier, 1994) and sheltering in crevices (Spanier and Almog-Shtayer, 1992) to prevent predatory attacks. Slipper lobsters suffer significantly less predation in the field than spiny lobsters or clawed lobsters when animals are tethered (thus minimizing the advantage of a giant escape circuit) (Barshaw *et al.,* 2003), which suggests that these alternative anti-predator strategies may compensate for the loss of the giant escape circuit. Previously, it was proposed that the behavioral (*e.g.,* digging) and morphological (*e.g.,* dorsoventral flattening) specializations of slipper lobsters preceded, and perhaps drove, neural changes—*i.e.,* loss of giant neurons (Faulkes, 2004). This hypothesis can be tested by examining spiny lobsters. Because the morphology of spiny lobsters is more similar to that of clawed lobsters and crayfish than to that of slipper lobsters, it was predicted that spiny lobsters should retain the escape-related giant neurons (Faulkes, 2004). An alternative hypothesis is that the loss of giant neurons preceded the morphological and behavioral specializations of slipper lobsters, and that changes to the nervous system drove some of the morphological and behavioral changes in the group, especially in the slipper lobsters. This hypothesis would be supported by the lack of giant neurons in spiny lobsters, because Palinuridae is the sister group to Scyllaridae.

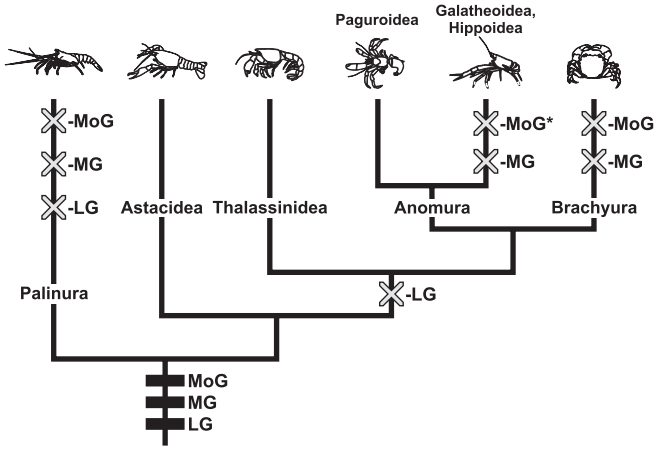


Figure 1. Phylogeny of reptantian decapods, based on data from Ahyong and O'Meally (2004), and distribution of escape-related giant neurons. The presence of medial giant (MG), lateral giant (LG), and motor giant (MoG) neurons is the ancestral condition for reptantians, a conclusion based on the presence of these neurons in non-reptantian shrimp and prawns (Johnson, 1924, 1926; Heuser and Doggenweiler, 1966; Xu and Terakawa, 1999), and in non-decapod syncarids (Silvey and Wilson, 1979). The LGs are absent in mud shrimp (Thallasinidea) (Turner, 1950) and hermit crabs (Anomura: Paguroidea) (Chapple and Hearney, 1976; Paul, 1991). Squat lobsters (Anomura: Galatheoidea) and sand crabs (Anomura: Hippoidea) have lost the MGs and LGs; the MoG is either absent (Wilson and Paul, 1987) or present as a putative non-giant homolog (Sillar and Heitler, 1985). There is no evidence for the giant escape circuit in true crabs (Brachyura) (Wiersma, 1961). Palinurans do not possess LGs, MG, or MoGs (Faulkes, 2004; this study). Animals not to scale.

Surprisingly, there is little evidence for or against the presence of giant neurons in spiny lobsters, even though spiny lobsters are well studied, and when present, the MGs and LGs are conspicuous because they are by far the largest neurons in decapod nervous systems. Reports of short-latency (~5 ms) tailflips (Newland *et al.,* 1992) and observations of the form of tailflips (Jacklyn and Ritz, 1986) suggest that the giant neurons are present. Jacklyn and Ritz (1986) also reported that *Ibacus peronii* had LGs (Jacklyn and Ritz, 1986), but later research did not support this observation (Faulkes, 2004). There is no anatomical evidence that spiny lobsters have escape-related giant neurons. References in the literature to giant neurons of spiny lobster are not referring to the giant escape circuit (*e.g.,* MGs, LGs, MoGs), but to abdominal fast flexor motor neurons in general (Warren and Rubin, 1978) and walking leg neurons (Villegas and Sanchez, 1991). Ecological data do not provide any strong hints as to whether giant-mediated escape responses might be expected in spiny lobsters: in field experiments, the predation suffered by tethered spiny lobsters was almost exactly intermediate between the high predation mortality of clawed lobsters and the minimal mortality of slipper lobsters (Barshaw *et al.,* 2003).

Here, we show that the spiny lobster species *Panulirus argus* (Latreille, 1804) does not have the giant neurons associated with escape responses. This work has previously appeared in abstract (Faulkes and Varghese, 2004; Espinoza *et al.,* 2005).

Materials and Methods

Specimens of *Panulirus argus* (Latreille, 1804), a spiny lobster, were purchased from Keys Marine Laboratory, Long Key, Florida, and housed in the Coastal Studies Laboratory at South Padre Island, Texas. The lobsters' carapace length ranged from 6 to 9 cm. As a positive control, all

techniques were also performed on Louisiana red swamp crayfish, *Procambarus clarkii* (Girard, 1852). The crayfish were purchased from Carolina Biological Supply and housed individually in freshwater tanks. Lobsters and crayfish were anesthetized by chilling on ice for at least 30 min. They were then dissected in chilled seawater (lobster) and freshwater saline (crayfish). The abdomen was removed at the thoracic-abdomen joint and pinned ventral side up onto a dish lined with Sylgard (Dow Corporation) and filled with crab saline. The ventral exoskeleton was detached and removed to expose the ventral nerve cord. The blood vessels and connective tissue were carefully removed, leaving the isolated nerve cord.

For sectioning, each nerve cord of six ganglia was cut into smaller segments. To prepare the nerve cord for sectioning, it was fixed in formalin overnight under a fume hood. The following morning the tissue was removed from the formalin and dehydrated in an alcohol series. The tissue was placed in a vial of a 1:1 mixture of xylene and 100% ethanol for 20 min, and solid pieces of Paraplast (wax) were added every 2 h until the wax would not dissolve. After being kept on top of a warm oven overnight, the tissue was put through a series of Paraplast replacements every 2 h. Then all the Paraplast was taken out and replaced with melted wax. The tissue was then embedded by quickly removing it from the vial, replacing it in a sample dish, and adding additional wax. Once solidified, the wax was taken out of the dish and a razor blade was used to carve a trapezoid shape around the tissue. The tissue was then sectioned (5–10 μm) with a microtome and stained with toluidine blue O epoxy tissue stain (Electron Microscopy Sciences, Catalog #14950) or hematoxylin and eosin. The tissue was then sealed with Permount and viewed under a microscope. Nerve cords from seven specimens of *P. argus* and four of *Procambarus clarkii* were sectioned.

The dorsal branch of nerve 3 ($N3_d$) was located and used for backfilling (Pitman *et al.,* 1972; Altman and Tyrer, 1980). Backfills were made by immersing the cut end of $N3_d$ in a small petrolatum well containing 2 mol l^{-1} cobalt chloride solution, and refrigerated overnight. The cobalt chloride was precipitated using ammonium sulfide, fixed in formalin, dehydrated in a progressive ethanol series (80%, 90%, 100% twice), cleared in methyl salicylate, and inspected under a light microscope. Twenty-four successful backfills (*i.e.,* showing multiple cell bodies) were made from nine *P. argus* individuals. Additionally, a further 29 backfills were made from seven recently deceased individuals. Although these fills were inferior in quality and rarely filled any cell bodies, they showed the fast flexor axons in the cord, which were examined for characteristics that typify the MoG (Mittenthal and Wine, 1978). The results from these additional fills were consistent with those from the freshly dissected individuals.

Photographs were taken with a digital camera and assembled into the final figures by using Corel Photo-Paint ver. 12 and Corel Draw ver. 12 (Corel Corporation).

Results

No large axons were visible in the dorsal region of abdominal nerve cord sections of *Panulirus argus* (Fig. 2A). In crayfish, the medial giant (MG) and lateral giant (LG) axons were clearly visible in the dorsal area of the nerve cord (Fig. 2B). Indeed, these large axons can be seen in an unstained crayfish nerve cord under a stereo light microscope. Although individual fast flexor motor neuron axons of the dorsal branch of nerve 3 ($N3_d$) are easily visible under a light microscope in *P. argus,* no MG and LG interneurons were visible in the spiny lobster nerve cord.

The fast flexor motor neurons of *P. argus* were found in three clusters in each of the anterior five abdominal ganglia (Fig. 3A–D, Fig. 4A), as they are in crayfish (Fig. 3E, Fig. 4B; Selverston and Remler, 1972; Mittenthal and Wine,

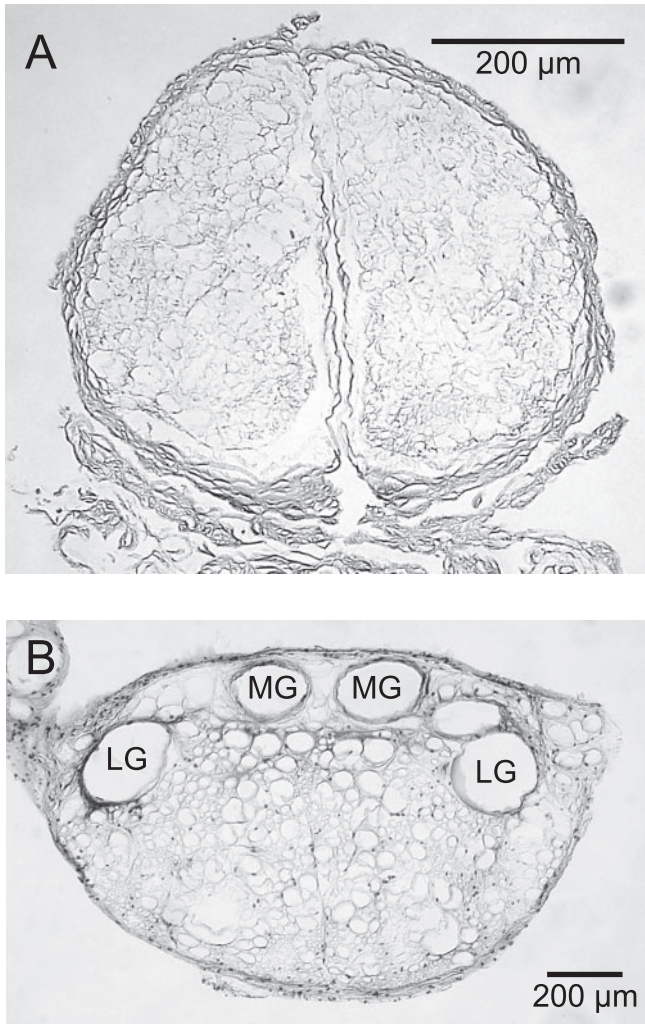
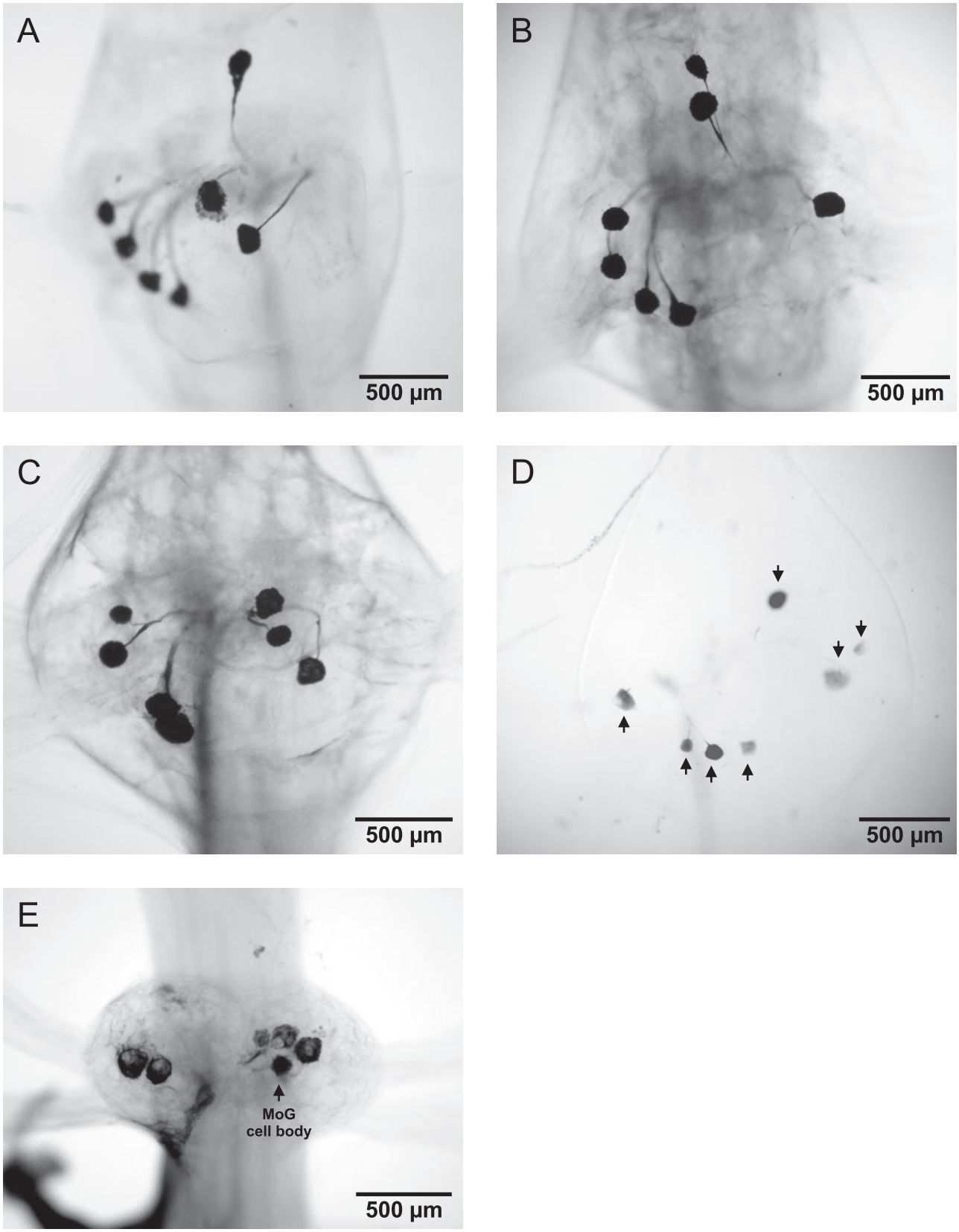


Figure 2. Cross sections of abdominal nerve cords. (A) Section between fourth and fifth abdominal ganglia in *Panulirus argus.* (**B**) Section between third and fourth abdominal ganglia in *Procambarus clarkii,* showing prominent motor giant (MG) and lateral giant (LG) axons.


A
500 µm
B
500 µm
C
500 µm
D
500 µm
E
MoG
cell body
500 µm

1978) and slipper lobsters (Faulkes, 2004): the flexor medial contralateral (FMC) cluster, the flexor posterior ipsilateral (FPI) cluster, and the flexor anterior contralateral (FAC) cluster (Mittenthal and Wine, 1978). Although the axons of all three clusters were found in one nerve ($N3_d$), the cell bodies of the FMC and FPI clusters were located in the ganglion anterior to a given nerve, and the cell bodies of the FAC cluster were located in the ganglion posterior to it.

In *P. argus,* the FMC cluster in all abdominal ganglia contained only three neurons due to the absence of the MoG (right clusters in Fig. 3A–D), whereas there are four FMC neurons in crayfish (right cluster in Fig. 3E; Mittenthal and Wine, 1978). No neurons backfilled though $N3_d$ in *P. argus* showed any of the characteristic features of the MoG (Fig. 5A, B). The crayfish MoG has several anatomical specializations that allow it to be easily recognized (Mittenthal and Wine, 1978). First, the MoG axon has a distinctly more medial projection than any other fast flexor motor neuron (Fig. 5C). Second, the MoG axon has numerous small processes in the connective between ganglia, where it makes electrical synapses with the MGs and LGs, and has no processes within the ganglion itself (Fig. 5D; Mittenthal and Wine, 1973). All fast flexor axons exit the ganglion in a single tight bundle. Note that even partial fills that do not reach the cell bodies could provide evidence of the MoGs.

The FPI cluster contained four motor neurons in all the abdominal ganglia of *P. argus* (left-hand clusters of cell bodies in Fig. 3A–D), including the fifth (Fig. 3D). This is similar but not identical to crayfish. The FPI cluster in crayfish contains four motor neurons in the four most anterior abdominal ganglia, but only three in the fifth abdominal ganglion (Mittenthal and Wine, 1978).

The FAC cluster is the most variable cell cluster both within the abdominal segments of a single species (Mittenthal and Wine, 1978) and across species (Wilson and Paul, 1987; Paul, 1991). Although characterizing the serial variation of the FAC cluster was not the aim of this project, it was visibly present in *P. argus* (Fig. 5A) and had a morphology similar to that of crayfish (Fig. 4B).

Although backfills are capricious (a tendency that seems to be exacerbated for freshwater crustaceans and small neurons), many fills made for this project were extremely high quality, with high contrast between filled cells and other tissue, and little blebbing or other distortion (Figs. 3–5). All five abdominal ganglia yielded at least two putatively complete fills of both clusters. The FPI cluster allowed the failure rate of backfills to be estimated. Using a sample of 20 backfills from six individuals (all fills performed by the same authors), 13 out of 20 FPIs showed the expected four cells bodies. Twelve out of 20 backfills of the FMC showed three cell bodies, as expected if the MoG is absent. Given the similar proportion of putatively complete fills in the two clusters, it is difficult to argue that there are actually four neurons in the FMC cluster in *P. argus* but that only three were seen because backfills do not always fill every cell and no FMC fill was complete.

Discussion

We found that spiny lobsters of the species *Panulirus argus* lack the three major sets of giant neurons responsible for fast-start escape responses, namely the medial giant (MG), lateral giant (LG), and motor giant (MoG) neurons. There is a common perception that demonstrating something to be absent is more difficult than showing it to be present, but whether such a demonstration is hard or easy depends mainly on whether the target sought is rare, small, or similar to other objects (Pasquerello, 1984). Giant neurons are the neural equivalent of the elephants in the room: it is easy to show that they are there, and easy to show that they are not. The non-giant fast flexor motor neuron pool in *P. argus* and *Procambarus clarkii* indicates that much of the rest of the abdominal flexor system is highly conserved (although not identical) across taxa, which aids detection of these differences.

Given that there is no anatomical evidence of escape-related giant neurons, how can physiological evidence of fast starts be explained? Previously, short-latency tailflips (~5 ms) in *Jasus lalandii* (H. Milne-Edwards, 1837) suggested that the giant escape circuit is present in spiny lobsters (Newland *et al.,* 1992). Whether such short latencies are possible in spiny lobsters remains to be confirmed, but it may be possible to reconcile such apparently fast responses with the lack of escape-related giant neurons. Although short response times have been viewed as impossible without giant neurons (Reichert and Wine, 1983),

Figure 3. Backfills of the dorsal branch of the third abdominal nerve showing cell bodies of fast flexor motor neurons located in ganglion anterior to the nerve: flexor posterior ipsilateral (FPI) cluster on the left; flexor medial contralateral (FMC) cluster on the right. In this and all subsequent figures, the filled nerve is positioned on the left. (**A**) Abdominal ganglion 1, slightly rotated, in *Panulirus argus.* (**B**) Abdominal ganglion 2 in *P. argus.* (**C**) Abdominal ganglion 3 in *P. argus;* fill from same individual as A. (**D**) Abdominal ganglion 5 in *P. argus;* arrows indicate cell bodies. Note that there are four cell bodies in the FPI cluster, whereas there are only three in this cluster in crayfish (Mittenthal and Wine, 1978). (**E**) Abdominal ganglion 2 in *Procambarus clarkii,* showing four cell bodies in the FMC cluster. The motor giant neuron (MoG) cell body was identified by tracing the axon back through the connective (out of focus in this view; see Figure 5 for examples of the distinctiveness of the MoG axon). The FPI cluster is incompletely filled; four cells are expected (Mittenthal and Wine, 1978).

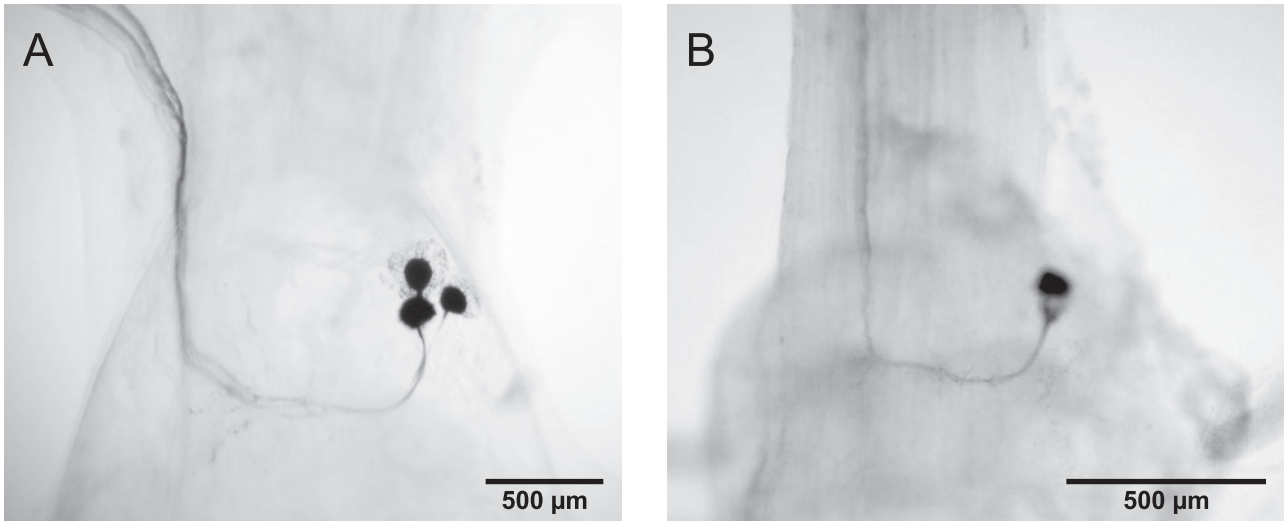


Figure 4. (**A**) Backfill of the dorsal branch of the third abdominal nerve in *Panulirus argus,* showing the flexor anterior contralateral (FAC) cluster in abdominal ganglion 2. (**B**) Incomplete backfill of the dorsal branch of the third abdominal nerve in *Procambarus clarkii,* showing one FAC motor neuron (of three) in abdominal ganglion 3 with characteristic "hook" projection pathway (Mittenthal and Wine, 1978).

non-giant tailflips can be almost as fast as giant-mediated ones if stimulated by a predatory strike rather than an experimenter's tap (Herberholz *et al.,* 2004). The mechanisms responsible for this difference in non-giant response times between natural and artificial stimuli are unknown. It is also possible that spiny lobster species in the genus *Jasus* have the giant escape circuit, while *P. argus* does not. Indeed, in squat lobsters, important differences in abdominal fast flexor neurons occur within a family (Sillar and Heitler, 1985; Wilson and Paul, 1987). Given that the giant escape circuit is absent in representative species in both Palinuridae (this study) and Scyllaridae (Faulkes, 2004), it is more parsimonious to assume that the giant escape circuit was lost once in the palinuran infraorders rather than repeatedly throughout the group. Further, preliminary data indicate that *J. edwardsii* (Hutton, 1875) also lacks the escape-related giant neurons (Z. Faulkes, unpubl. data).

Panulirus argus was expected to have the giant escape circuit because its morphology and behavior are generally similar to those of other decapods that are known to have the giant escape circuit, particularly clawed lobsters, and are dissimilar to scyllarids in these respects. Spiny lobsters and clawed lobsters have a similar body shape, whereas scyllarids are strongly dorsoventrally flattened. Both spiny lobsters and clawed lobsters use defensive weapons against predators (antennae and claws, respectively) (Barshaw *et al.,* 2003), whereas scyllarids use clinging, armor, non-giant tailflipping, and digging for predator avoidance (Jacklyn and Ritz, 1986; Spanier *et al.,* 1991; Spanier and Almog-Shtayer, 1992; Barshaw and Spanier, 1994; Barshaw *et al.,* 2003; Faulkes, 2004). Thus, the loss of giant neurons in palinurans was not a response by the behavioral and morphological specializations characteristic of slipper lobsters. Instead, the results here suggest that the loss of the giant escape circuit may have been important in selecting for lineages with effective new anti-predator strategies to compensate for the loss of fast escape responses.

The loss of escape-related giant neurons in palinurans generally is unexpected, because rapid escape responses are typically thought to be under strong selection pressure (Eaton, 1984). That giant-mediated tailflips significantly reduce successful predation attempts on juvenile crayfish (Herberholz *et al.,* 2004) supports this view. On the other hand, the observation that, under experimental conditions, spiny lobsters suffer less predation than clawed lobsters (Barshaw *et al.,* 2003) indicates that the interplay between different anti-predator mechanisms is complex, and the relative importance of some of these mechanisms may need to be reappraised. For example, communal sheltering in dens may be an example of an anti-predator strategy that has greater significance in the ecology of spiny lobsters because of the absence of giant neurons (Marx and Herrnkind, 1985; Eggleston *et al.,* 1990; Briones-Fourzán *et al.,* 2003). *Panulirus argus* is often gregarious, which can aid in predator deterrence (Herrnkind *et al.,* 1975; Atema and Cobb, 1980; Kanciruk, 1980)—triggerfish, for example, are attracted to individual lobsters that are separated from groups (Herrnkind *et al.,* 1975). Similarly, the exoskeleton is thicker and heavier in spiny lobsters than in clawed lobsters (Barshaw *et al.,* 2003), and the spiny lobster antennae are effective weapons, more so than claws (Kanciruk, 1980; Barshaw *et al.,* 2003). Thus, alternative anti-predator strategies may compensate for the slower response time that is predicted.

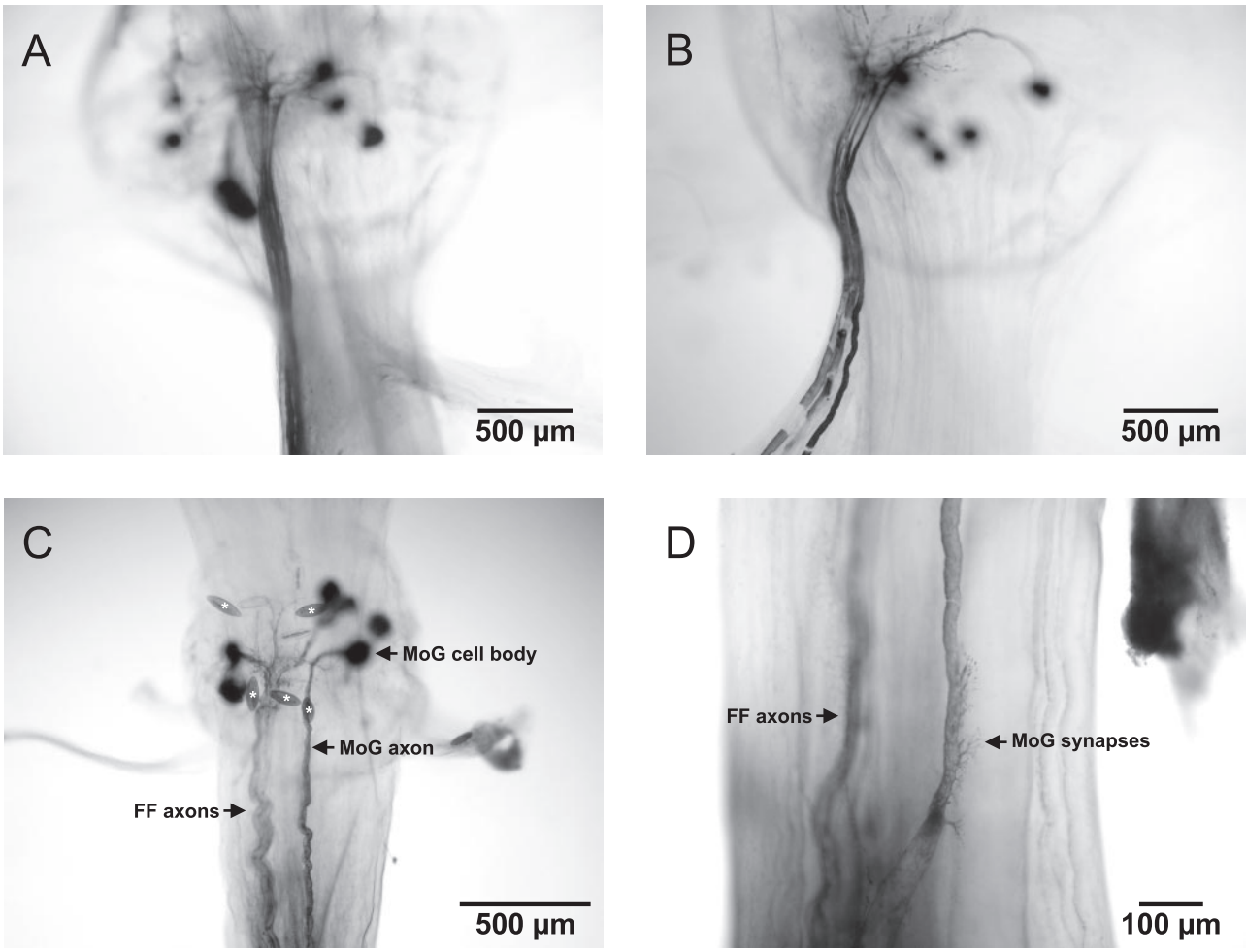


Figure 5. Backfills of the dorsal branch of the third abdominal nerve. (**A**) Abdominal ganglion 3 in *Panulirus argus;* different view of the same fill in Figure 3C. (**B**) Abdominal ganglion 2 in *P. argus.* Note that A and B show tight clumping of axons in the connective, with none showing any of the small processes in the connective indicative of synaptic connections between the MoG and giant interneurons (Mittenthal and Wine, 1973). (**C**) Abdominal ganglion 1 in *P. clarkii,* showing the two separate axon pathways: one medial pathway containing the motor giant (MoG) axon, and the other lateral pathway containing other fast flexor axons. (Small oval bodies marked with asterisks believed to be parasites.) (**D**) Connective between abdominal ganglia 1 and 2 in *Procambarus clarkii* (different individual than C), showing the short processes where the MoG synapses with one of the giant interneurons.

The loss of escape-related giant neurons in palinurans is an example of "reverse evolution" (Porter and Crandall, 2003) and may qualify as a disaptation: a feature that is less advantageous for survival than the ancestral condition (Montgomery and Clements, 2000). The crustacean giant escape circuit serves as an excellent model to study the evolution of neural circuits: fast escape responses have clear implications for survival; the neurons are widespread across many species (Fig. 1); and as this study shows, there is more variation in the giant escape circuit across taxa than previously expected, and few explanations—mechanistic, developmental, adaptive, or otherwise—have been proposed to explain this variation.

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Literature Cited

Ahyong, S. T., and D. O'Meally. 2004. Phylogeny of the Decapoda Reptantia: resolution using three molecular loci and morphology. *Raffles Bull. Zool.* **52:** 673–693.

Altman, J. S., and N. M. Tyrer. 1980. Filling selected neurons with cobalt through cut axons. Pp. 373–402 in *Neuroanatomical Techniques,* N. J. Strausfeld and T. A. Miller, eds. Springer-Verlag, Berlin.

Atema, J., and J. S. Cobb. 1980. Social behavior. Pp. 409–450 in *The Biology and Management of Lobsters,* J. S. Cobb and B. F. Phillips, eds. Academic Press, New York.

Barshaw, D. E., and E. Spanier. 1994. Anti-predator behaviors of the Mediterranean slipper lobster, *Scyllarides latus. Bull. Mar. Sci.* **55:** 375–382.

Barshaw, D. E., K. L. Lavalli, and E. Spanier. 2003. Offense *versus* defense: responses of three morphological types of lobsters to predation. *Mar. Ecol. Prog. Ser.* **256:** 171–182.

Briones-Fourzán, P., V. Castañeda-Fernández de Lara, E. Lozano-Álvarez, and J. Estrada-Olivo. 2003. Feeding ecology of the three juvenile phases of the spiny lobster *Panulirus argus* in a tropical reef lagoon. *Mar. Biol.* **142:** 855–865.

Chapple, W. D., and E. S. Hearney. 1976. The morphology of the fourth abdominal ganglion of the hermit crab: a light microscope study. *J. Morphol.* **144:** 407–420.

Eaton, R. C. 1984. *Neural Mechanisms of Startle Behavior.* Plenum Press, New York.

Edwards, D. H., W. J. Heitler, and F. B. Krasne. 1999. Fifty years of a command neuron: the neurobiology of escape behavior in the crayfish. *Trends Neurosci.* **22:** 153–160.

Eggleston, D. B., R. N. Lipcius, D. L. Miller, and L. Coba-Cetina. 1990. Shelter scaling regulates survival of juvenile Caribbean spiny lobster *Panulirus argus. Mar. Ecol. Prog. Ser.* **62:** 79–88.

Espinoza, S. Y., A. Breen, N. Varghese, and Z. Faulkes. 2005. Loss of escape-related neurons in spiny lobster (*Panulirus argus*). *Integr. Comp. Biol.* **45:** 1129 (Abstract).

Faulkes, Z. 2004. Loss of escape responses and giant neurons in the tailflipping circuits of slipper lobsters, *Ibacus* spp. (Decapoda, Palinura, Scyllaridae). *Arthropod Struct. Dev.* **33:** 113–123.

Faulkes, Z. 2005. Do shovel-nosed lobsters shovel with their noses? *Integr. Comp. Biol.* **45:** 994.

Faulkes, Z. 2006. Digging mechanisms and substrate preferences of shovel nosed lobsters, *Ibacus peronii* (Decapoda: Scyllaridae). *J. Crustac. Biol.* **26:** 69–72.

Faulkes, Z., and N. Varghese. 2004. No escape: loss of escape-related giant neurons in spiny lobsters (*Panulirus argus*). Program No. 89.4. Neuroscience 2004 [Online: Abstract Viewer/Itinerary Planner]. Society for Neuroscience, Washington, DC. (Abstract.)

Furshpan, E. E., and D. D. Potter. 1959. Transmission at the giant motor synapses of the crayfish. *J. Physiol.* **145:** 289–325.

Govind, C. K., and F. Lang. 1976. Growth of lobster giant axons: correlation between conduction velocity and axon diameter. *J. Comp. Neurol.* **170:** 421–434.

Hale, M. E., J. H. Long, M. J. McHenry, and M. W. Westneat. 2002. Evolution of behavior and neural control of the fast-start escape response. *Evolution* **56:** 993–1007.

Heitler, W. J., and K. Fraser. 1993. Thoracic connections between crayfish giant fibres and motor giant neurones reverse abdominal pattern. *J. Exp. Biol.* **181:** 329–333.

Herberholz, J., M. M. Sen, and D. H. Edwards. 2004. Escape behavior and escape circuit activation in juvenile crayfish during prey-predator interactions. *J. Exp. Biol.* **207:** 1855–1863.

Herrnkind, W. F., J. Van DerWalker, and L. Barr. 1975. Population dynamics, ecology and behavior of spiny lobsters, *Panulirus argus,* of St. John, U.S.V.I. IV. Habitation, patterns of movement and general behavior. *Nat. Hist. Mus. Los Angel. Cty. Sci. Bull.* **20:** 31–45.

Heuser, J. E., and C. F. Doggenweiler. 1966. The fine structural organization of nerve fibers, sheaths, and glial cells in the prawn, *Palaemonetes vulgaris. J. Cell Biol.* **30:** 381–403.

Holmes, W. 1942. The giant myelinated nerve fibres of the prawn. *Philos. Trans. R. Soc. Lond.* **B231:** 293–314.

Jacklyn, P. M., and D. A. Ritz. 1986. Hydrodynamics of swimming in scyllarid lobsters. *J. Exp. Mar. Biol. Ecol.* **101:** 85–99.

Johnson, G. E. 1924. Giant nerve fibres in crustaceans with special reference to *Cambarus* and *Palaemonetes. J. Comp. Neurol.* **36:** 323–365.

Johnson, G. E. 1926. Studies on the functions of the giant fibers of crustaceans, with special reference to *Cambarus* and *Palaemonetes. J. Comp. Neurol.* **42:** 19–33.

Jones, C. M. 1988. The biology and behaviour of bay lobsters, *Thenus* spp. (Decapoda: Scyllaridae), in northern Queensland, Australia. Ph.D. thesis, University of Queensland.

Kanciruk, P. 1980. Ecology of juvenile and adult Palinuridae (spiny lobsters). Pp. 59–96 in *The Biology and Management of Lobsters. Vol. 2, Ecology and Management,* J. Cobb and B. Phillips, eds. Academic Press, New York.

Krasne, F. B., and D. H. Edwards. 2002. Crayfish escape behavior: lessons learned. Pp. 3–22 in *Crustacean Experimental Systems in Neurobiology,* K. Wiese, ed. Springer-Verlag, Berlin.

Krasne, F. B., and J. J. Wine. 1984. The production of crayfish tailflip escape responses. Pp. 179–211 in *Neural Mechanisms of Startle Behavior,* R. C. Eaton, ed. Plenum Press, New York.

Laura, E. 2006. The tactile-stimulated startle response of tadpoles: acceleration performance and its relationship to the anatomy of wood frog (*Rana sylvatica*), bullfrog (*Rana catesbeiana*), and American toad (*Bufo americanus*) tadpoles. *J. Exp. Zool. A Comp. Exp. Biol.* **305A:** 348–362.

Marx, J. M., and W. F. Herrnkind. 1985. Macroalgae (Rhodophyta: *Laurencia* spp.) as habitat for young juvenile spiny lobsters, *Panulirus argus. Bull. Mar. Sci.* **36:** 423–431.

Mittenthal, J. E., and J. J. Wine. 1973. Connectivity patterns of crayfish giant interneurons: visualization of synaptic regions with cobalt dye. *Science* **179:** 182–184.

Mittenthal, J. E., and J. J. Wine. 1978. Segmental homology and variation in flexor motoneurons of the crayfish abdomen. *J. Comp. Neurol.* **177:** 311–334.

Montgomery, J., and K. Clements. 2000. Disaptation and recovery in the evolution of Antarctic fishes. *Trends Ecol. Evol.* **15:** 267–271.

Newland, P. L., D. Cattaert, D. M. Neil, and F. Clarac. 1992. Steering reactions as adaptive components of the tail-flip in the spiny lobster *Jasus lalandii. J. Exp. Biol.* **164:** 261–282.

Otis, T. S., and W. F. Gilly. 1990. Jet-propelled escape in the squid, *Loligo opalescens:* concerted control by giant and non-giant motor axon pathways. *Proc. Natl. Acad. Sci. USA* **87:** 2911–2915.

Pasquerello, T. 1984. Proving negatives and the paranormal. *Skeptical Inquirer* **8:** 259–270.

Paul, D. H. 1991. Pedigrees of neurobehavioral circuits: tracing the evolution of novel behaviors by comparing motor patterns, muscles, and neurons in members of related taxa. *Brain Behav. Evol.* **38:** 226–239.

Pitman, R. M., C. D. Tweedle, and M. J. Cohen. 1972. Branching of central neurons: intracellular cobalt injection for light and electron microscopy. *Science* **176:** 412–414.

Porter, M. L., and K. A. Crandall. 2003. Lost along the way: the significance of evolution in reverse. *Trends Ecol. Evol.* **18:** 541–547.

Reichert, H., and J. J. Wine. 1983. Coordination of lateral giant and non-giant systems in crayfish escape behavior. *J. Comp. Physiol. A* **153:** 3–15.

Selverston, A. I., and M. P. Remler. 1972. Neural geometry and

activation of crayfish fast flexor motoneurons. *J. Neurophysiol.* **35:** 797–814.

Sillar, K. T., and W. J. Heitler. 1985. The neural basis of escape swimming behaviour in the squat lobster *Galathea strigosa.* I. Absence of cord giant axons and anatomy of motor neurons involved in swimming. *J. Exp. Biol.* **117:** 251–269.

Silvey, G. E., and I. S. Wilson. 1979. Structure and function of the lateral giant neurone of the primitive crustacean *Anaspides tasmaniae. J. Exp. Biol.* **78:** 121–136.

Spanier, E., and G. Almog-Shtayer. 1992. Shelter preferences in the Mediterranean slipper lobster: effects of physical properties. *J. Exp. Mar. Biol. Ecol.* **164:** 103–116.

Spanier, E., D. Wehs, and G. Almog-Shtayer. 1991. Swimming of the Mediterranean slipper lobster. *J. Exp. Mar. Biol. Ecol.* **145:** 15–31.

Turner, R. S. 1950. Functional anatomy of the giant fiber system of *Callianassa californiensis. Physiol. Zool.* **23:** 35–41.

Villegas, G. M., and F. Sanchez. 1991. Periaxonal ensheathment of lobster giant nerve fibres as revealed by freeze-fracture and lanthanum penetration. *J. Neurocytol.* **20:** 504–517.

Walker, J. A., C. K. Ghalambor, O. L. Griset, D. McKenney, and D. N. Reznick. 2005. Do faster starts increase the probability of evading predators? *Funct. Ecol.* **19:** 808–815.

Warren, R. H., and R. W. Rubin. 1978. Microtubules and actin in the giant nerve fibres of the spiny lobster, *Panulirus argus. Tissue Cell* **10:** 687–697.

Wiersma, C. A. G. 1947. Giant nerve fiber system of the crayfish. a contribution to comparative physiology of synapse. *J. Neurophysiol.* **10:** 23–38.

Wiersma, C. A. G. 1961. Reflexes and the central nervous system. Pp. 241–277 in *Physiology of Crustacea,* T. H. Waterman, ed. Academic Press, New York.

Wilson, L. J., and D. H. Paul. 1987. Tailflipping of *Munida quadrispina* (Galatheidae): conservation of behavior and underlying musculature with loss of anterior contralateral flexor motoneurons and motor giant. *J. Comp. Physiol. A* **161:** 881–890.

Wine, J. J. 1984. The structural basis of an innate behavioural pattern. *J. Exp. Biol.* **112:** 283–319.

Wine, J. J., and F. B. Krasne. 1972. The organization of escape behaviour in the crayfish. *J. Exp. Biol.* **56:** 1–18.

Wine, J. J., and F. B. Krasne. 1982. The cellular organization of crayfish escape behavior. Pp. 241–292 in *Neural Integration and Behavior,* D. C. Sandeman and H. L. Atwood, eds. Academic Press, New York.

Xu, K., and S. Terakawa. 1999. Fenestration nodes and the wide submyelinic space form the basis for the unusually fast impulse conduction of shrimp myelinated axons. *J. Exp. Biol.* **202:** 1979–1989.