No escape: Loss of escape-related giant neurons in spiny lobsters (*Panulirus argus*)

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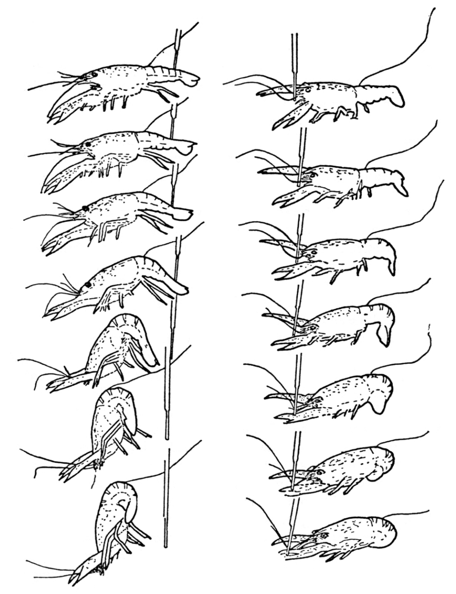
Introduction

The crayfish escape circuit

Crayfish (Astacidea) have a well-studied circuit of giant neurons that mediate escape responses (Edwards et al. 1999, Wine and Krasne 1972, 1982). Key neurons in this circuit are the medial giant interneurons (MGs), lateral giant interneurons (LGs), and fast flexor motor giant neurons (MoGs).

The MGs spike in response to sudden sensory input to the cephalothorax (e.g., a tap). The MGs cause the MoGs in all abdominal segments to flex, which cause the animal to move away from the stimulus.

The LGs spike in response to abdominal taps. The LGs do not synapse with the MoGs in the three posterior abdominal segments, so only the front of the tail flexes, causing the crayfish to pitch its posterior end into the water column.



LG-mediated (**left**) and MG-mediated (**right**) tailflips. From Wine & Krasne (1972).

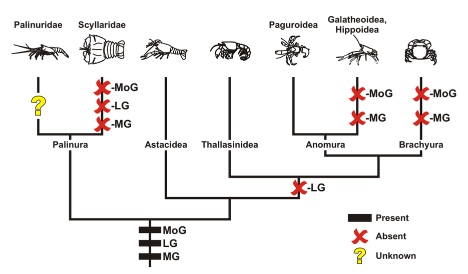
Slipper lobsters lack the escape circuit

This circuit is ancestral to reptantian decapods, because non-reptantian species (e.g., shrimp, prawns) have MGs and LGs. Several decapod crustacean taxa, however, have lost some or all of the circuit (Paul 1991, Sillar and Heitler 1985, Wilson and Paul 1987), including slipper lobsters (Palinura: Scyllaridae) (Faulkes 2004, Wiersma 1961). This is an independent evolutionary loss relative to previously described losses. Such independent losses can test hypotheses about the evolution of nervous systems. The following competing hypotheses could explain the loss of the escape response.

Hypothesis 1: Behavioural changes preceded neural changes. Slipper lobsters are heavily armoured, dorsoventrally flattened, and conceal themselves by digging into sand. This hypothesis suggests that the loss of the escape circuit was an adaptation to digging (Faulkes 2004). Escapes by a buried animal may be either ineffective due to the lack of movement imposed by sand, or deleterious because it could draw attention to a concealed individual.

Hypothesis 2: Neural changes preceded behavioural changes. This hypothesis suggests that the loss of the escape circuit was not an adaptation to digging.

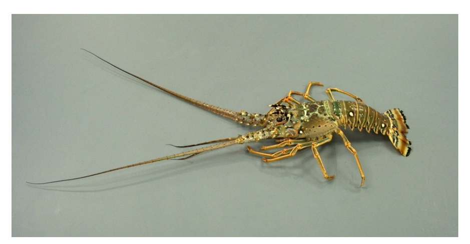
Slipper lobster (*Ibacus peronii*). Photo: David Paul.



Distribution of escape-related giant neurons in reptantian decapod crustaceans. Phylogeny based on Scholtz & Richter (1995) and Schram (2001).

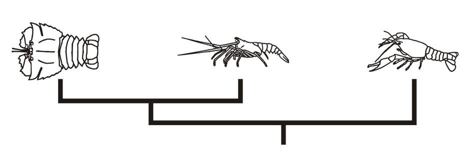
Do spiny lobsters have the escape circuit?

Spiny lobsters (Palinura: Palinuridae) are the closest relatives to the slipper lobsters, but there is no anatomical evidence of the escape circuit in this group. Spiny lobsters can test the hypothesis that digging behaviour preceded the loss of the escape circuit. If spiny lobsters **have** the escape circuit, it would be consistent with behavioural changes driving neural changes. If spiny lobsters do **not** have the escape circuit, it indicates that losing the escape circuit preceded (and perhaps drove) slipper lobsters' behavioural and morphological changes. There are, however, reasonable grounds for predicting either the presence or absence of the escape circuit in spiny lobsters.

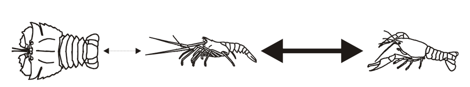


Spiny lobster (*Panulirus argus*).

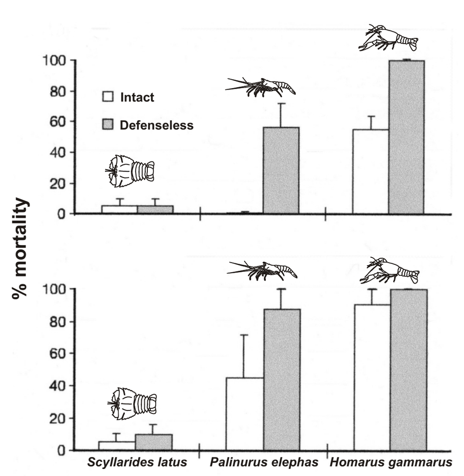
Phylogeny: The close relationship between the spiny lobsters and the slipper lobsters suggest that spiny lobsters should **lack** the escape circuit.



Morphology and behaviour: Spiny lobsters' overall body and behaviour is more similar to crayfish and clawed lobsters than slipper lobsters. These factors suggest that spiny lobsters should **have** the escape circuit.



Predation pressure: Spiny lobsters experience less predation than clawed lobsters, but more than scyllarids, under experimental field conditions (Barshaw et al. 2003). Spiny lobsters are intermediate to the slipper lobsters and clawed lobsters in this regard. Thus, these data do not strongly support either hypothesis.



Mortality after 4 (**top**) and 24 (**bottom**) hours of predation on size matched, tethered lobsters. "Defenseless" = slipper lobster, legs bound; spiny lobsters, antennae removed; clawed lobsters, claws removed. From Barshaw et al. (2003).

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Methods

Spiny lobsters (*Panulirus argus*) were purchased from Keys Marine Lab, Florida, and housed in the Coastal Studies Lab, South Padre Island, Texas. Lobsters were anaesthetized by chilling before being dissected. The abdomen was dissected, and the ventral nerve cord was removed. We examined nerve cords for large dorsal axons (i.e., LGs and MGs) under a stereo microscope. Nerve cords were embedded in paraffin, and sectioned on a microtome (5 µm slices). We searched for MoGs by staining the fast flexor motor neurons by cobalt backfilling the dorsal branch of the third nerve ($N3_d$) of abdominal ganglia 1 through 5.

As a positive control, all techniques were also performed on Louisiana red swamp crayfish (*Procambarus clarkii*), purchased from a commercial supplier (Carolina Biological Supply).

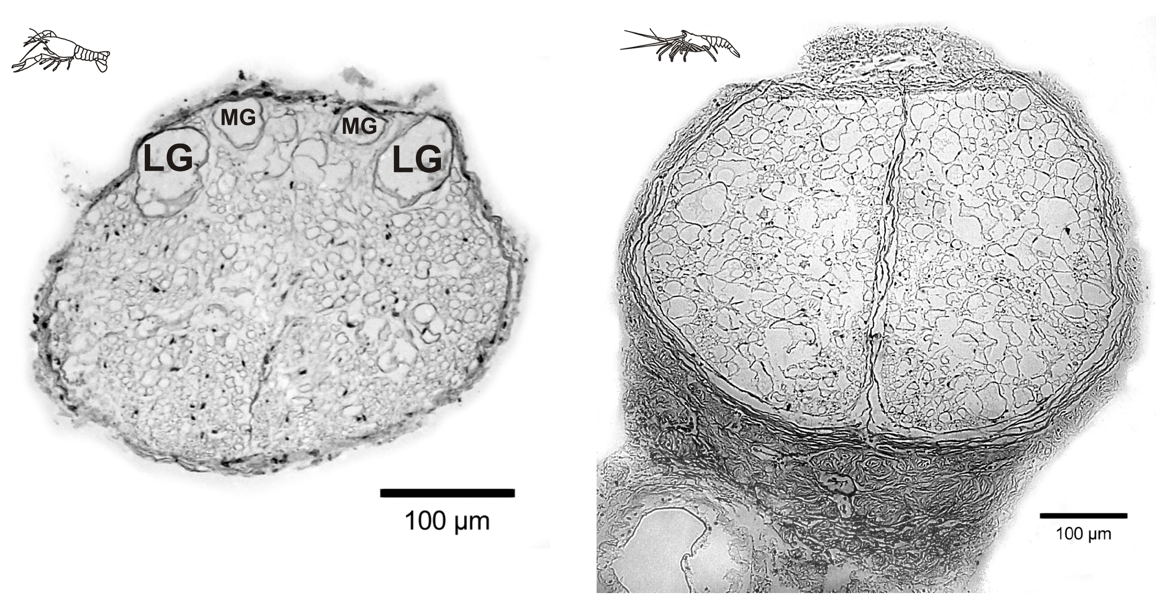
Results

Spiny lobsters lack MGs and LGs

In crayfish, the MG and LG axons are clearly visible in the dorsal area of the nerve cord under low power on a dissecting microscope. Furthermore, large axon profiles are also visible in thin sections cut between the abdominal ganglia.

No large axons or axon profiles are visible in the dorsal nerve cord of spiny lobsters using either technique.

Nerve cords of *P. clarkii* (**left**) and *P. argus* (**right**) viewed under a stereo microscope.

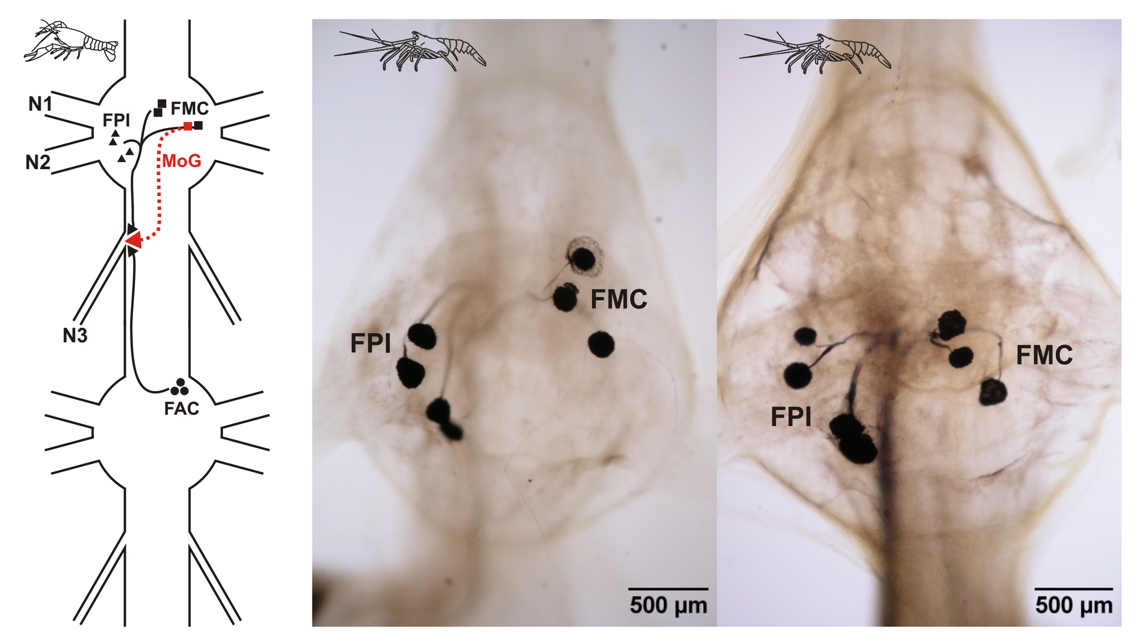


Thin sections (5 µm) of abdominal nerve cords between fifth and sixth of *P. clarkii* (**left**) and *P. argus* (**right**).

Spiny lobsters lack MoGs

In crayfish, there are three clusters of fast flexor motor neuron cell bodies: the flexor medial contralateral (FMC), flexor posterior isilateral (FPI), and flexor anterior contralateral (FAC) cluster (Mittenthal & Wine 1978). The MoG cell body is one of four neurons in the FMC cluster. The MoG is distinct from other fast flexors: (a) the axon projects more medially than other fast flexor motor neuron axons; (b) the axon has small processes in the nerve cord, where the MoG forms electrical synapses with the MGs and LGs .

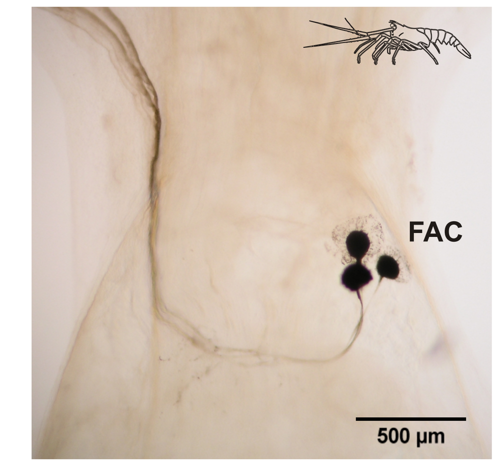
In spiny lobsters, the FMC cluster contains three cell bodies, none of which show any of the anatomical specializations that characterize the MoG. All of the fast flexor axons exit the ganglion in a single tight bundle, and none of the fast flexor axons have processes in the nerve cord.



Map of fast flexor motor neurons in crayfish abdominal ganglia (**left**). Red = MoG. Two $N3_d$ cobalt fills in *P. argus* (**center, right**) showing complete fills of FMC and FPI clusters.

Spiny lobsters retain the FAC cluster

Although describing the FAC cluster in detail was not a goal of this project, this group of cells is present in *P. argus*. The FAC cluster is more variable than the other fast flexor motor neurons. It has been lost at least twice: once in the sand crab superfamily (Hippoidea), and once in the squat lobster species *Munida quadrispina* (Wilson and Paul 1987). Curiously, another squat lobster species, *Galathea strigosa*, still has the FAC cluster (Sillar and Heitler 1985).

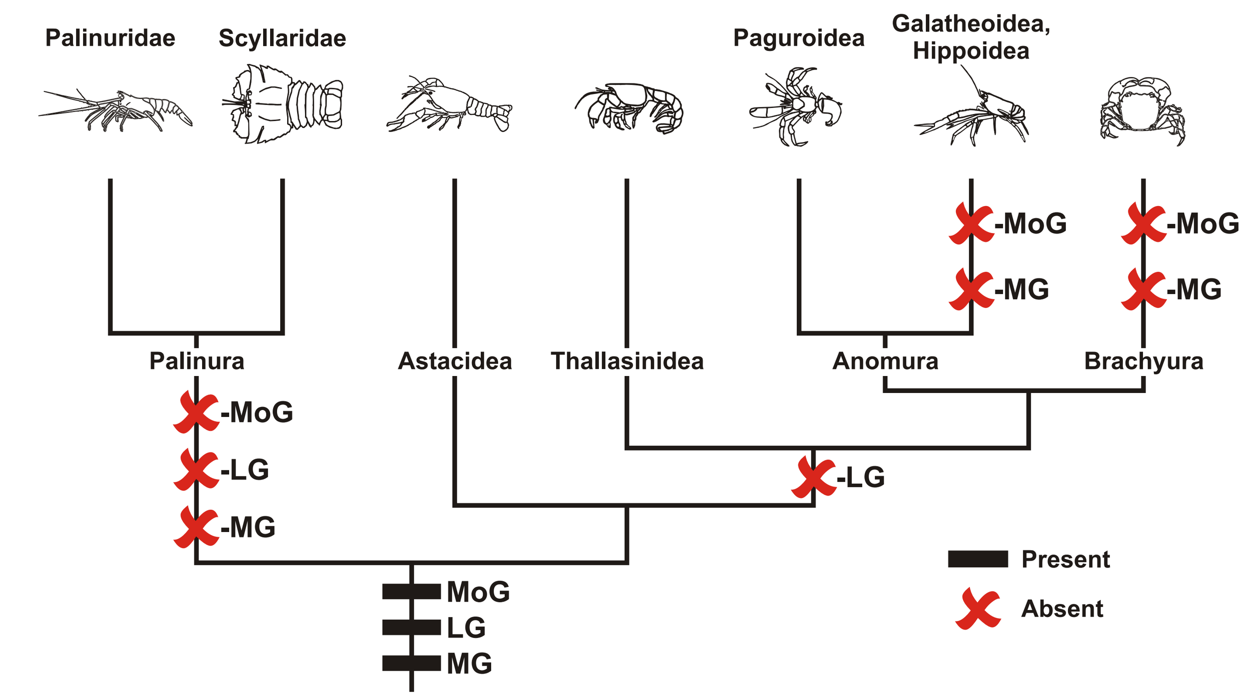


Cobalt fill of $N3_d$ in *P. argus*.

Discussion

Spiny lobsters lack the giant neurons necessary for rapid escape responses. Thus, the loss of the escape circuit preceded the divergence of the palinuran and scyllarid families, and may have driven the morphological and behavioural specializations of slipper lobsters. The loss of these neurons cannot be a secondary adaptation in response to scyllarids' predator avoidance strategies.

The repeated loss of an apparently adaptive behaviour is unexpected because escape responses are thought to be under strong selection pressure. Nevertheless, both spiny lobsters (*Palinurus elephas*) and slipper lobsters (*Scyllarides latus*) are at lower predation risk from triggerfish than clawed lobsters (*Homarus gammarus*) of similar size (Barshaw et al. 2003), which is consistent with the presence of escape circuit in clawed lobsters only. Palinurans have a heavier exoskeleton than clawed lobsters, indicating that thick armour is a more effective defence against predators than claws and rapid escape responses in some situations.



Distribution of escape-related giant neurons in reptantian decapod crustaceans, incorporating the results of the present study. Phylogeny based on Scholtz & Richter (1995) and Schram (2001).

Acknowledgements and errata

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Authors after the first are listed alphabetically by last name.

The published abstract does not list Alana Breen and Sandra Espinoza as co-authors because they contributed to this work after the abstract deadline.

The published abstract title erroneously gives the species name as *Palinurus argus*, rather than *Panulirus argus*.

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