MOTO-IGERT:
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Sensory Innervation and Proprioception of Fish Fins.

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INTRODUCTION

Pectoral fins act as primary propulsors for many species of fish and function in rhythmic swimming as well as arrhythmic behaviors such as maneuvering. Little work, however, has focused on the role of pectoral fins in mechanosensation. Many pectoral-fin swimmers live in complex structural or flow environments where sensory feedback may be particularly important for modulating movement patterns. With this work we ask whether the pectoral fins act not only to generate force for movement but also to take in sensory information about the fin ray bending. Mechanosensation of the pectoral fins has been observed in sea robins that probe the substrate for prey (Bardach and Case, 1965) and in sharks (Lowenstein, 1956). We examine mechanosensation in the bluegill sunfish (*Lepomis macrochirus*), a species that has been widely studied as a model for pectoral fin-based propulsion (e.g. Gibb et al., 1994; Lauder et al., 2006). We examined the morphology of ray nerves, and observed the physiological responses of the nerve fibers to step-and-hold and sinusoidal bending stimuli.

METHODS

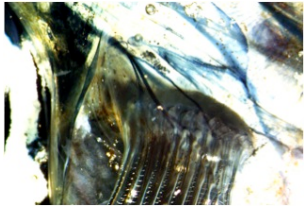
Immunocytology
Methods for immunocytology follow those found in Thorsen and Hale [2007], with the exception of the primary antibody used. A mouse anti-neurofilament marker (3A10, Sigma-Aldrich) was used as the primary antibody. The secondary antibody used was a goat antimouse antibody conjugated with either rhodamine or fluorescein. Fluorescent images of the labeled nerves were collected with a Zeiss LSM confocal microscope (Thornwood, NY).

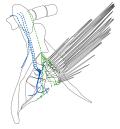
Sudan Black B Staining
Fins were stained with a modified version of the methods from Filipski and Wilson (2004) and Song and Parenti (1995). Fish were fixed in 10% formalin solution for one week, followed by a one week rinse in deionized water. The fins were removed and placed in a trypsin solution until muscles became translucent. Specimens were gradually dehydrated to 70% ethanol and placed in a 30% Sudan Black B solution overnight. After rehydrating, the fins were placed back in trypsin until nerves were clearly visible and then soaked in 0.5% KOH solution overnight. This was followed by storage in glycerol.

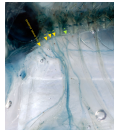
Neurophysiology
The pectoral fins of adult bluegills were excised and placed in extracellular solution to create a fictive fin preparation. The fin rays were held in place by clamping the fin membrane near the base of the fin. The fin was then stimulated with bending stimuli. Physiological responses were recorded from nerves innervating the fin rays on the medial side of the fin using a multi-unit extracellular recording electrode. The methods for stimulation are detailed in panel 3.

RESULTS

1. The pectoral fin receives extensive innervation from four nerve branches.

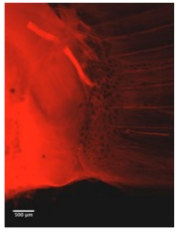


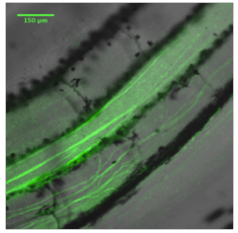


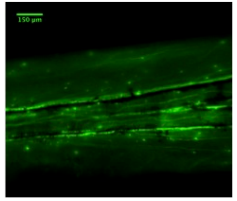


Images of nerves extending from the central nervous system distally to the base of the fin rays.

2. Many nerve fibers project into the fin rays and surrounding membrane.





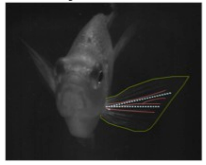


Upper left: pectoral fin nerves branch to innervate each of the fin rays.

Upper right: nerve fibers extend distally through the fin ray core and adjacent membrane.

Lower right: nerve fibers terminate along the length of the fin ray and in the adjacent membrane.

3. A fictive preparation was developed to record from the fin rays nerves while the fin is being actuated.



Bluegill with fin extended showing rays.

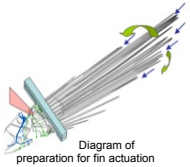
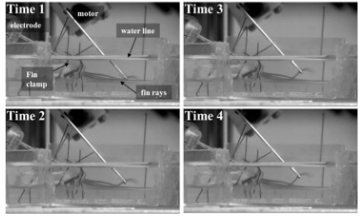
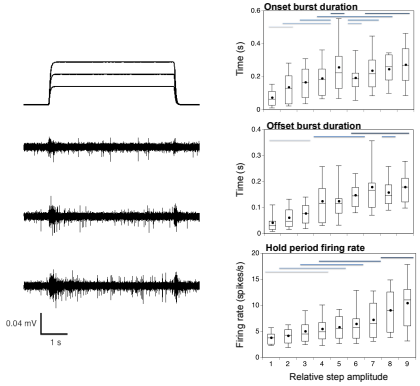


Diagram of preparation for fin actuation

Images of fin ray bending in physiology experiments

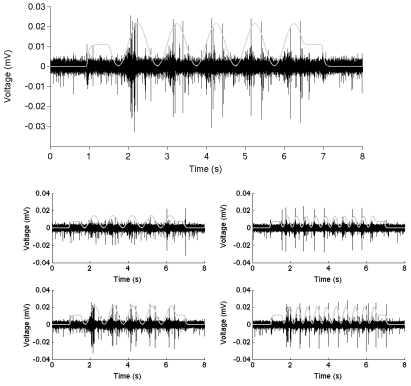


4. Activity of fin ray nerves in response to step-and-hold bending.



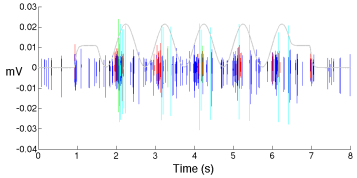
Multiunit nerve recordings show responses specific to the bending of individual rays. Left: responses to step-and-hold bends of increasing amplitude. Right: multiunit responses change as a function of step amplitude (1 step = 1.44mm)

5. Activity of fin ray nerves in response to sinusoidal bending.



Nerves fibers innervating the fin rays and membrane showed a consistent, localized response to sinusoidal bending. Multiunit responses varied with the amplitude and frequency of stimuli.

6. Spike sorting of multiunit activity shows differing single unit activity during sinusoidal bend period.



Separate afferent classes respond to specific aspects of the sinusoidal bend stimulus.

CONCLUSIONS

Fin rays of bluegill sunfish are heavily invested with nerve fibers that we believed to be sensory due to their distal locations.

Activity recorded from fin ray nerves demonstrates that these nerves are sensitive to fin ray bending.

Step-and-hold stimuli resulted in strong activity in fin ray nerves when the associated rays were deflected and returned to their resting position. The duration of the activity was related to the amplitude of the deflection, with greater deflections resulting in longer bursts of activity. Increases in the amplitude of step-and-hold stimuli also resulted in an increase in the activity rate of the nerve during the hold period of the stimulation.

Sinusoidal oscillations of the fin rays resulted in phasic activity in fin ray nerve. Spike sorting indicates that particular nerve cells are active at specific points during the movement cycle.

Together our data indicate that the fin rays of bluegill sunfish receive substantial mechanosensory information during fin ray bending that reflect the extent and dynamics of fin ray movement. We suggest that mechanosensation is a fundamental function of the fin rays typically thought of as locomotor and should be considered in analyses of fin-based motor control, functional morphology and evolution.

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