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Sensory Nerve Endings of Highly Mobile Structures in Two Marine Teleost Fishes

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Summary. With the use of a whole mount silver impregnation technique, sensory nerve endings were located in the connective tissue at the base of the modified pectoral fin ray in the gurnard, *Aspitrigla cuculus*, and within the perichondrium of the barbel in the goatfish, *Mullus surmuletus*. The location of these endings and their planar receptory fields in such highly mobile structures, suggests that the sensory endings are proprioceptive in nature and that they are associated in monitoring the positional state of the modified pectoral fin ray and barbel, respectively, during voluntary movement. This investigation addresses itself to the general problem of proprioception in teleost fishes and provides histological evidence for the presence of proprioceptive nerve endings.

A. Introduction

Fishes do not have muscle spindles. Proprioceptors of a simpler nature than muscle spindles have been described both histologically and electrophysiologically in only elasmobranch fishes. The so-called "terminaison en pinceau", first described by Poloumordwinoff (1898), is a brush-like ending with varicosities found in the intramuscular connective tissue of the pectoral fins of rays. In a classic study, Fessard and Sand (1937) were able to elicit tension-sensitive recordings upon manipulation of the pelvic fins in *Raja*. Wunderer (1908) found coiled corpuscular endings at the bases of fins in various shark species. Lowenstein (1956) investigated these endings and showed that they were slowly adapting mechanoreceptors, functioning as second-order proprioceptors since as the fin was bent, compression or stretching of the connective tissue on which the endings lie resulted in receptor discharge. The corpuscular endings were also found later to be present in subcutaneous tissues external to the myotomal muscles in the shark (Bone, 1964). These two sensory endings represent the simplest known vertebrate proprioceptors.

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Only two histological studies exist to my knowledge in which proprioceptive endings were supposedly found in teleost fishes and illustrations published. Pansini (1888) describes simple free endings in the tendon of the dorsal fin muscle in the seahorse, *Hippocampus* Rafinesque, 1810. Ciaccio (1890–91) describes sensory endings resembling tendon organs in the caudal tendon of the tench, *Tinca tinca* (Linnaeus, 1758) and in the goldfish, *Carassius vulgaris* (Nielsson, 1832). I have not been able to duplicate their results with the use of a very reliable silver impregnation technique.

Recently, Ballintijn and Bamford (1975) have detected evidence of proprioceptive feedback in the respiratory neurones of the carp, *Cyprinus carpio* (Linnaeus, 1758). Roberts (1969) has observed rhythmical sensory discharges upon sinusoidal bending of the myotomal musculature in the gurnard, *Aspitrigla cuculus* (Linnaeus, 1758). In both electrophysiological investigations, no histological elucidation was provided of the proprioceptive endings from which the recordings were made.

Information of both a histological and electrophysiological nature in teleost fishes would assist in the understanding of many intriguing questions regarding the neural control of locomotion, respiration, and feeding.

The scarcity of histological information led me to investigate structures in teleost fishes where a high degree of motor control seemed necessary. My reasoning was that sensory endings of a proprioceptive nature might be needed in the positioning of such highly mobile structures.

The following investigation centers on two such highly maneuverable structures, notably the modified pectoral fin rays of the gurnard and the barbel of the goatfish. The three modified pectoral fin rays can move independently in an arc of 180°. These fin rays are so mobile that early studies claimed that locomotion was their main function. The goatfish barbel is actually comprised of two bilaterally symmetrical cartilaginous rods which can maneuver independently and whip around rapidly in searching the substrate for food.

B. Methods

Freshly killed specimens of the gurnard (*Aspitrigla cuculus*) and the goatfish (*Mullus surmuletus*) (Linnaeus, 1758) were collected from the English Channel off of Plymouth, England.

Sixty-four pectoral girdles of the gurnard and thirty barbels were used in this study. Supra-vital methylene blue was administered on some of the fresh specimens in order to initially locate the pattern of innervation present. The dissected material was pinned on paraffin slips, floated in a pool of 0.1% methylene blue and either counterstained with 1% osmic acid or fixed with a saturated ammonium molybdate solution. The rest of the specimens were fixed for ten days or longer in 10% unbuffered formalin. A longer fixation time revealed better results.

In order to insure proper fixation for maximal staining with silver, the connective tissue region around the joint capsules of the three modified pectoral fin rays of the gurnard was exposed and the cartilaginous rod of the goatfish barbel was carefully dissected out of its thick epidermis.

Nerve endings and their associated innervation patterns could be visualized using the Winkelmann and Schmitt silver impregnation technique (Winkelmann and Schmitt, 1957; Bone, 1972). Although this technique was originally developed for frozen mammalian tissue sections, it worked perfectly well for whole mount fish material.

Appropriate silver-stained nerve endings in connective tissue were run up through an alcohol-xylene dehydration series and mounted on microscope slides with Dammar resin for examination under the compound microscope.

Several dozen preparations of the receptory fields in the connective tissue of the gurnard joint capsule and within the perichondrium of the goatfish barbel were carefully teased out.

C. Results

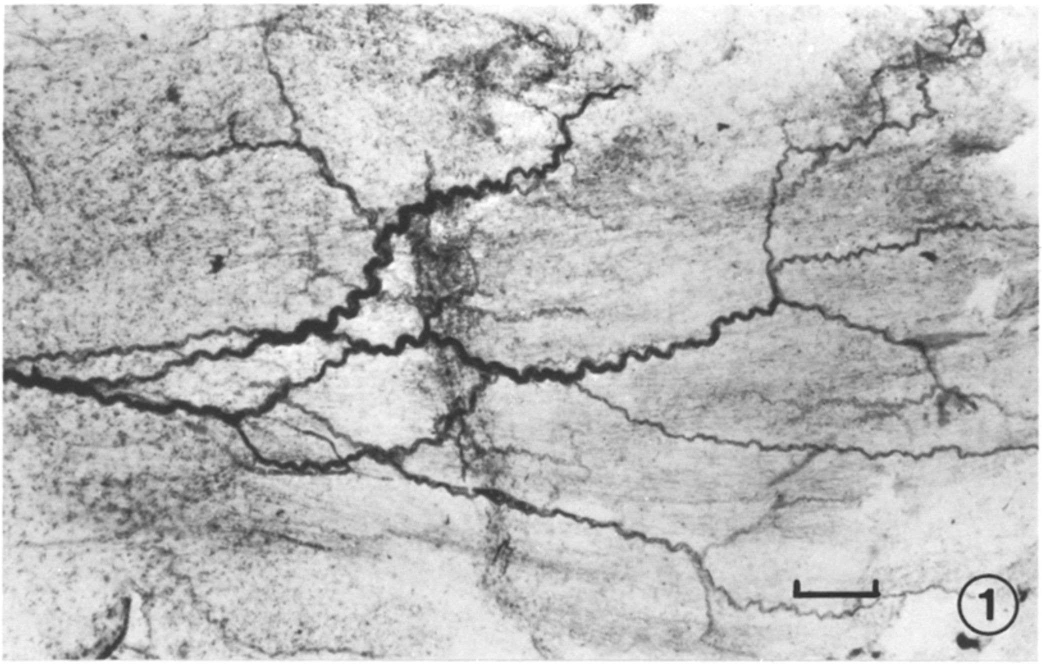
The gurnard modified pectoral fin ray is controlled by three pairs of extrinsic muscles and is innervated by a large branch from the third spinal nerve. The connective tissue at the base of the modified pectoral fin ray was overlain by the extrinsic fin ray musculature, the dermis containing an abundance of guanine crystals, and the epidermis.

Silver staining of the adductors superficialis and profundus and abductors superficialis and profundus controlling the fin ray did not reveal any easily discernible sensory endings analogous to muscle spindles found in the intramuscular connective tissue of higher vertebrates. There was no presence of intrinsic musculature in the fin ray itself. In addition, the connective tissue at the bases of the unmodified pectoral fin rays did not reveal any sensory endings, but one is cautious here in making any definitive statements regarding this apparent absence since an absence of evidence is not necessarily conclusive.

Varicose sensory nerve endings lying in connective tissue at the bases of the modified pectoral fin rays were found, however. These nerve endings arose from six to eight myelinated axons which together formed a large nerve trunk spreading out into a planar receptory field in a plane parallel to the direction of fin movement (Fig. 1). These receptory fields varied from 1,800–3,000 μm in length and 1,100–1,200 μm in width. The number of axons making up the trunk varied. Each axon branched several times forming numerous varicose endings. The varicosities found spaced along the endings varied between 1.0–2.5 μm in diameter (Fig. 2).

The goatfish barbel consisted of a central cartilaginous rod apposed by a large nerve trunk, the hyomandibular branch of the facial nerve (cranial nerve VII). There was no intrinsic musculature present within the barbel. The loose connective tissue of the dermis with its associated blood vessels and nerve fiber bundles surrounds the cartilaginous rod and nerve trunk which is, itself, enveloped by the thick epidermis (Sato, 1937a). The hyomandibular branch of the facial nerve sends the majority of its axons peripherally to innervate the chemo- and tactile receptor cells located within the epidermis.

Stripping off the epidermis and dermis revealed a closely adhering layer of perichondrium attached to the cartilaginous rod. Free simple sensory nerve endings within the perichondrium were revealed upon silver staining. The endings varied from 0.5–1.0 μm in diameter. No varicosities were observed. Each receptory field was composed of four to five myelinated axons which spread out within the perichondrium to terminate as free nerve endings. These endings branched out parallel to the long axis of the cartilaginous rod. Nerve endings from adjacent receptory fields overlapped to a certain extent (Fig. 3 and 4). The dimensions of the receptory field varied from 550–700 μm in length and 40–100 μm in width. No sensory endings were observed in the extrinsic muscles controlling the barbel using this silver impregnation technique.



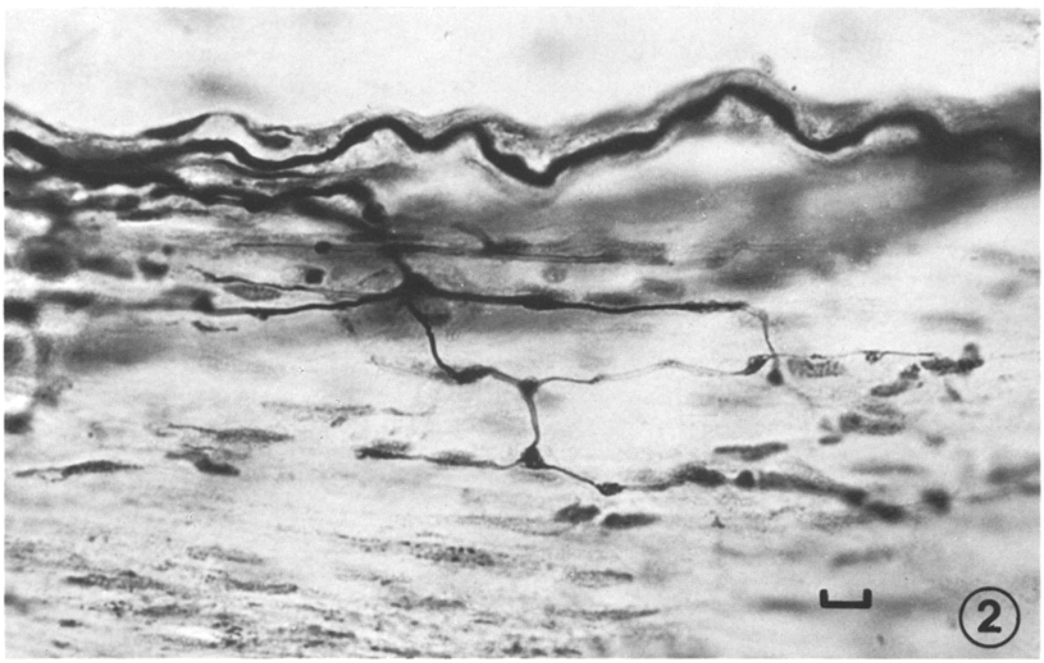


Fig. 1. Sensory receptory field of *Aspitrigla cuculus* (bar represents 100 µm)

Fig. 2. A varicose sensory nerve ending from a branch of the receptory field of *Aspitrigla cuculus* (bar represents 5.0 µm)

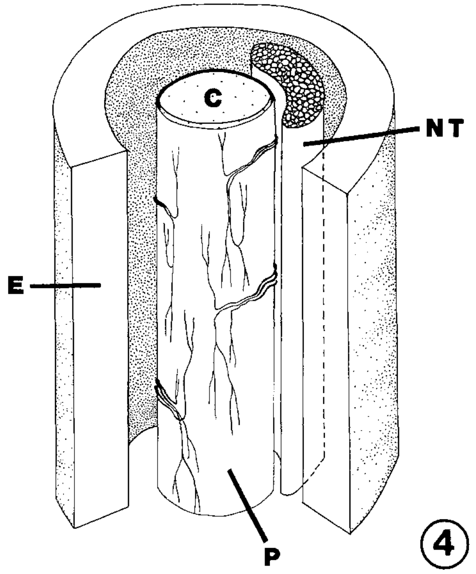


Fig. 3. Sensory receptory field from the perichondrium within the barbel of *Mullus surmuletus* (bar represents 50 μm)

Fig. 4. Simplified diagram illustrating section of the barbel in *Mullus surmuletus*. For purposes of clarity, the dermis has not been shown. *C* – cartilage, *E* – epidermis, *NT* – nerve trunk (hyomandibular branch of facial nerve), *P* – perichondrium

Both gurnard and goatfish receptory fields occurred within flat areas of connective tissue and were located at the boundaries between two different tissue layers. The gurnard receptory field was located between the extrinsic muscles and the bony elements of the joint capsule, while the goatfish receptory field was found between the cartilage of the rod and the loose connective tissue of the dermis.

D. Discussion

The "walking pectoral appendages" or modified pectoral fin rays of the gurnard have amazed investigators since the early 19th century. Virtually all of the studies deal with the role the distal ends of these fin rays play in chemical and tactile reception (Tiedeman, 1816; Morrill, 1895; Herrick, 1907; Belling, 1912; Scharrer, Smith and Palay, 1947; Scharrer, 1963; Bardach and Case, 1965). No mention is made as to how such a mobile structure can monitor its position in space.

Bone (1964) mentions that he has observed simple free sensory nerve endings at the base of the modified pectoral fin ray in gurnards. However, no illustrations were ever published. My results indicate that the endings are free sensory nerve endings with varicosities.

The joint capsule formed by the modified pectoral fin ray and scapula also exhibits an abundant vascular supply. It is interesting to speculate as to what extent the sensory endings participate in the control of the lumina of the vessels, if at all, or whether they merely serve in signaling joint capsular changes. Free sensory nerve endings were found lying within the perichondrium of the goatfish barbel. A similar result was reported in the lungfishes (Dipnoi) by Holmes (1967). He located free sensory nerve endings within the perichondrium of the pectoral and pelvic fins of the lungfish, *Protopterus*. These fins consist of axial cartilaginous rods and are capable of refined movements.

According to Poláček (1966), large numbers of receptors monitoring positional state usually accumulate in certain areas forming receptory fields. These receptory fields are invariably placed within flat areas of connective tissue at the boundary between two tissues capable of moving in relation to each other. Both *Aspitrigla* and *Mullus* display two-dimensional receptory fields which are located in areas subject to a great deal of movement. It is based on the localities in which the receptory fields of both *Aspitrigla* and *Mullus* lie, that I suggest that these endings act as proprioceptors.

As far as I am aware, the results obtained in this investigation are the first published photographs of sensory nerve endings in teleost fishes which appear to be proprioceptive in nature. Whether the endings are specifically joint, pressure, stretch, tension, or other type of proprioceptor remains unanswered. The location of these endings suggest that: 1) the sensory nerve endings found in *Aspitrigla* may act as joint receptors monitoring the position of the pectoral fin ray during movement and 2) the sensory nerve endings found lying within the perichondrium of *Mullus* may act as stretch receptors monitoring the degree of bending of the barbel.

Although sensory endings of a proprioceptive nature have been alluded to, neither these receptors nor any other sensory ending have been located in the intramuscular connective tissue of the extrinsic musculature controlling both the fin ray of the gurnard and the barbel of the goatfish which could analogize them to neuromuscular spindles of higher vertebrates. Since there does not appear to be any specialized receptor like the muscle spindle located within the muscles of teleost fishes (Barker, 1974), it becomes very difficult, if not impossible, to distinguish between sensory and motor endings solely through the use of silver impregnation in this region. It does appear likely, however, that proprioceptive endings of a less specialized nature than neuromuscular spindles occur within the muscle fibers of teleost fishes based on the electrophysiological data accrued by various investigators.

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