Odonatologica 10 (1): 29-37 *March 1, 1981*

THE STRUCTURE AND PHYSIOLOGY OF THE TARSO-PRETARSAL CHORDOTONAL ORGAN IN THE LARVA OF *ANAX IMPERATOR* LEACH (ANISOPTERA: AESHNIDAE)

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Received August 20, 1980

The tarso-pretarsal (TP) chordotonal organ is described. It is unusual in that only a small number (ca 8) of bipolar sensory cell bodies are present in the cellular strand. Each scolopidium encloses a single centriolar derivative. The cilium arises from a cross-striated ciliary root and has a 9 + 0 axoneme configuration running from base to apex of the centriolar derivative. A scolopale cell surrounds each cilium to form the intracellular scolopale, extracellular scolopale space and distal cap. No attachment cell is seen distally, instead each scolopidium appears in close association with the surrounding strand cells. Unidirectional sensory units were recorded from the TP organ. Most of these were sensitive to flexion. New names are proposed for some insect joint chordotonal organs to conform with crustacean terminology.

INTRODUCTION

Connective chordotonal organs occur at most of the moveable leg joints in both insects and crustaceans, and serve to monitor movement and position of the joints. Their distribution and anatomy have been fairly extensively studied in both groups and there is abundant information on the number of sensory cells in a wide range of receptors (cf. MILL, 1976, for crustaceans; WRIGHT, 1976, for insects). However, although the ultrastructure of a number of crustacean limb chordotonal organs has been studied (e.g. WHITEAR, 1960, 1962; MILL & LOWE, 1971, 1973; LOWE et al., 1973), there is comparatively little information on those of insects. This is also in marked contrast to the attention which has been given to those insect chordotonal organs associated with tympanal organs (e.g. GRAY &

PUMPHREY, 1958; GRAY, 1960; YOUNG, 1973) and Johnston's organ (e.g. UGA & KUWABARA, 1965; HOWSE & CLARIDGE, 1970; MASON & GABOURIAUT, 1973; CORBIÈRE-TICHANÉ, 1975).

The femoro-tibial organ (cf. Discussion for terminology) in the pro- and mesothoracic legs of the grasshoppers, *Chortophaga viridifasciata* and *Romalea microptera*, has been studied by MORAN et al. (1975) and SLIFER & SEKHON (1975), respectively; the tibio-tarsal organ in *Periplaneta americana* by YOUNG (1970); and the tarso-pretarsal organ in the water bug, *Notonecta glauca* by WIESE & SCHMIDT (1974). In addition, HOWSE (1968) has described the ultrastructure of the subgenual organ (a chordotonal organ which lies at the proximal end of the tibia) in *Periplaneta americana*, and FRIEDMAN (1972) that of the subgenual and adjacent 'intermediate' organ in the cricket, *Gryllus assimilis*. The subgenual and intermediate organs are not associated with a joint.

MATERIAL AND METHODS

Late instar larvae of *Anax imperator* Leach were used.

STRUCTURE

To expose the tarso-pretarsal organ, the tarsus was opened along its mid-ventral surface. Receptors were removed, for examination with phase contrast or Nomarski optics. For the ultrastructural study, the third tarsal segment was fixed in 2.5% glutaraldehyde (SABATINI et al., 1963) overnight. The preparation was washed in cacodylate buffer, post-fixed in 1% osmium tetroxide in veronal buffer (PALADE, 1952) and washed in veronal buffer. It was dehydrated in a series of alcohols and them embedded in Epon gradually, over a period of three days, using increasing proportions of Epon in the Epon: propylene oxide mixture.

Transverse sections were cut on a Cambridge ultramicrotome, placed on carbon-coated 100 mesh copper grids and stained with uranyl acetate followed by lead citrate (REYNOLDS, 1963) for examination in an AEI EM6B electron microscope.

PHYSIOLOGY

For the physiological study, a metathoracic leg was detached, placed on its side, and a 7 mm length of a 0.19 mm diameter pin passed through the base of the pretarsus (claw). The leg was anchored ventral side up, with staples, to a silicone elastomer (Sylgard 170A/B) platform in the bottom of a Perspex bath, leaving the second-third tarsal joint extending just beyond the platform

edge. A series of small holes was made on each side of the femur, and the ventral cuticle pulled away from the underlying tissues. The leg nerve was removed carefully from the surrounding tissues and placed on a pair of platinum hook electrodes. The electrical activity was amplified using a Grass P9 pre-amplifier, and displayed on one channel of an oscilloscope.

A hooked tungsten-wire guide was mounted on a micromanipulator. The third tarsus was moved about its proximal joint by moving the guide at approximately 45° to the horizontal, with the pin in the pretarsus running freely in the guide (cf. MILL & HARRIS, 1977, for further details). The movement of the guide was monitored by a displacement transducer (Intersonde DR68) attached to the micromanipulator, and the transducer output was displayed on the second channel of the oscilloscope. It should be noted that the transducer monitored the motion of the wire guide, and thus the monitor trace gives only an approximate indication of the movement about the second-third tarsal joint.

RESULTS

The tarso-pretarsal organ lies on the dorsal side of the third tarsal segment and runs obliquely from near the proximal end of this segment to a distal insertion between the claws (pretarsus) (Fig. 1). Methylene blue staining indicated about eight cell bodies; phase contrast the presence of the scolopidia (PILL, 1978).

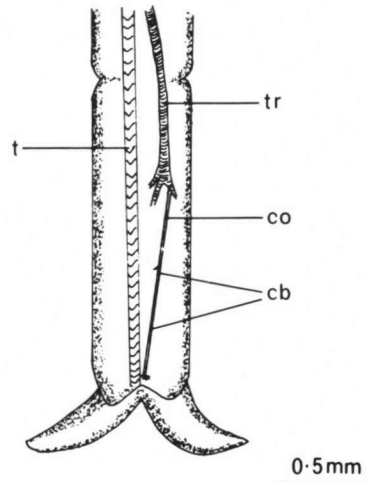

Fig. 1. Drawing showing the position of the tarso-pretarsal chordotonal organ. (cb, sensory cell bodies; co, chordotonal organ; t, tendon; tr, tracheole)

STRUCTURE

The receptor strand is oval in cross-section, measuring about 15x25 μm; it contains the bipolar sensory cells with their associated accessory cells, strand cells and connective tissue. Each of the bipolar sensory cells contains a pronounced, cross-striated ciliary root, which is circular in cross-section (Fig. 2a, b). This ends distally in a basal body, just beyond which the dendrite tapers abruptly and gives rise to a modified cilium (the centriolar derivative). The basal body comprises a centriolar-like formation, with triplets of microtubules (Fig. 2c), giving rise to the axoneme. This has a 9 + 0 arrangement of paired microtubules (Fig. 2d).

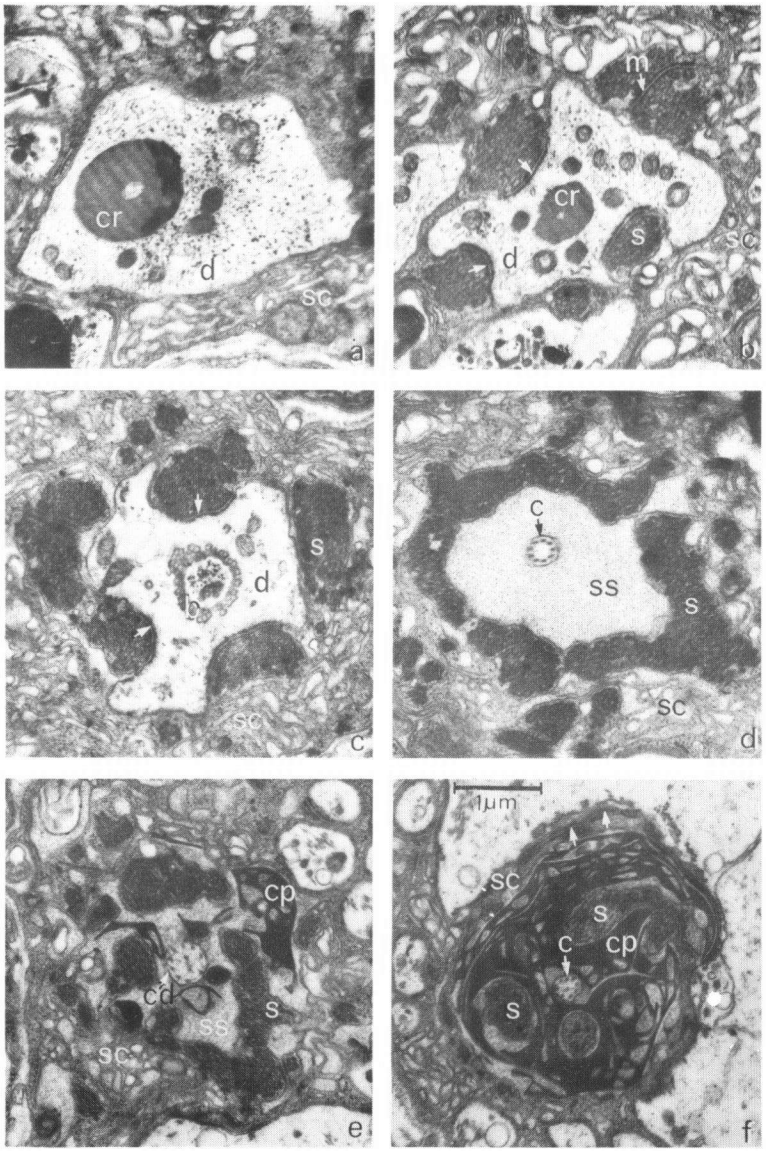

Fig. 2. Transverse sections (electron micrographs) of a scolopidium (proximal to distal): (a-c) Dendritic region; — (d) Ciliary region; — (e-f) Subterminal region. (b, basal body; c, cilium; cd, ciliary dilation; cp, cap; cr, ciliary root; d, dendrite; m, mesaxon; s, scolopale; sc, scolopale cell; ss, scolopale space; arrows, desmosomes)

One microtubule of each pair has an electron-dense core and bears a pair of side arms. Distally, the cilium dilates and then tapers. In the ciliary dilation, the diameter of the axoneme increases and, in the central region, there is some fibrous material (Fig. 2e). Distal to the ciliary dilation each element of the axoneme consists of two microtubules: the electron-dense core and the side arms are absent.

A scolopale cell extends distally from the region of the ciliary root. It contains a number of mitochondria and, in the ciliary root region, intracellular, electron-dense material around a matrix of microtubules. Most of this material is in the form of five rods (Fig. 2b, c), which increase in size distally and partially coalesce to form a cylinder surrounding the dendrite in the region of the ciliary segment (Fig. 2d). This electron-dense structure is called the scolopale. Patches of scolopale material are also distributed throughout the scolopale cell. Desmosomes occur at the points of attachment between the dendrite and the scolopale cell in the region of the five scolopale rods (Fig. 2b, c). The mesaxon can be seen particularly clearly in Figure 2b. This is the double membrane formed where the scolopale cell, which encircles the sensory apparatus, adheres to itself. Distally the scolopale encloses a scolopale space (1.1 - 2.6 μm in diameter), through which the cilium runs (Fig. 2d).

In the region of the ciliary dilation the scolopale breaks up into separate rods again and the scolopale space is gradually occluded distally as the cap is approached (Fig. 2e). The cap is an electron-dense structure of uniform consistency, containing lacunae (Fig. 2f). It completely surrounds the distal end of the scolopale cell and its greatest diameter is about 3 μm. No specialized attachment cell was seen, and the cap appeared to be embedded directly in the strand.

PHYSIOLOGY

In the extended (rest) position the sensory cells remained silent. However, there was a marked sensory discharge as the pretarsus was flexed (Figs. 3-5). There was some indication of variation in the adaptation rates of the different units, and the largest unit recorded was phaso-tonic. During a sequence of increase in its firing frequency and, at this angle, it did not adapt completely fire at a joint angle of about 35° to the horizontal, but adapted rapidly when the joint was held at 45°. Subsequent movement to 70° produced a marked increase in its firing frequency and, at this angle, it dit not adapt completely after a period of several seconds. Movement to 90° caused a further slight phasic response in the unit. It thus shows some degree of position sensitivity. The tonic component failed to adapt completely after about 8 seconds of maintained flexion at 90°. With repetitive flexion and extension, there was

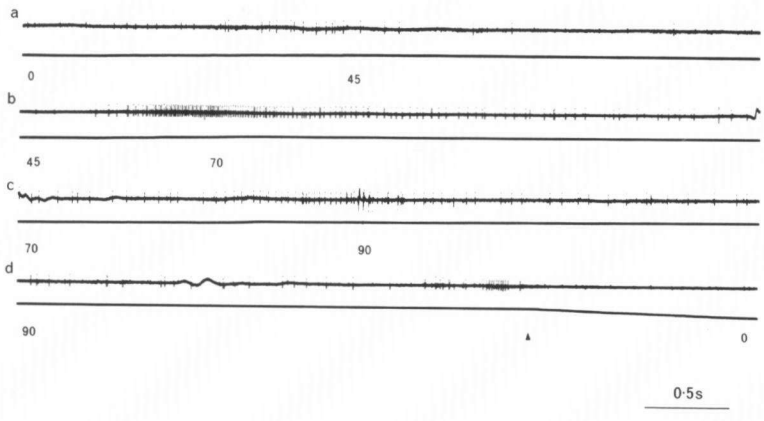

Fig. 3. Continuous recording of step-wise flexion of the third tarsal segment, and subsequent extension. Upper trace, tarso-pretarsal organ activity; lower trace, flexion (upwards) and extension (downwards) of the third tarsal segment. The degree of flexion is indicated, with 0° representing the horizontal position, 90° the vertical position.

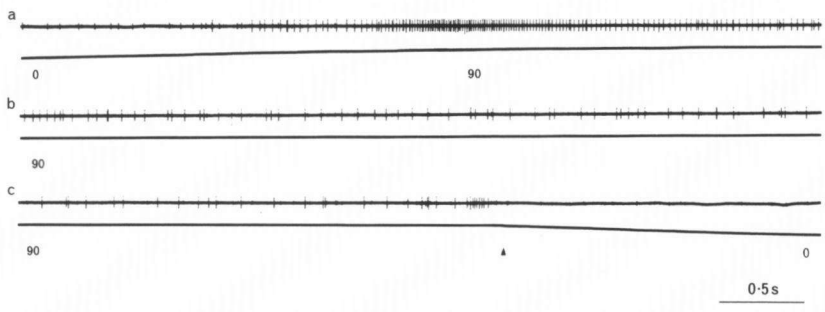

Fig. 4. Continuous recording in which 90° flexion is maintained for about 8 s. Details as in Figure 3.

Fig. 5. Repetitive flexions and extensions of the third tarsal segment through 90°. Details as in Figure 3.

some adaptation of the phasic response of the large unit (Fig. 5).

There was little or no activity during joint extensions, except for one fairly small unit which fired at the very beginning of extension. Indeed, the firing of this unit appears to precede extension in Figures 3d, 4c and 5, but this is almost certainly a result of the arrangement of the wire guide (cf. Methods).

DISCUSSION

It has been suggested that the limb chordotonal organs associated with joint movement and position in insects should be brought into line with crustacean terminology and named after the joints which they monitor (HUGHES & MILL, 1974; MILL, 1976). Thus the femoral chordotonal organ should be called the femoro-tibial (FT) chordotonal organ; the tarsal chordotonal organ located in the third tarsal segment should be renamed the tarso-pretarsal (TP) chordotonal organ; while the tibio-tarsal (TT) chordotonal organ should retain its present name.

In orthopterans, the FT organ of the pro- and mesothoracic legs has two scoloparia lying in the proximal region of the femur, a proximal one with 200-400 sensory cells, and a distal one with about 50 sensory cells (BURNS, 1974; MORAN et al., 1975; SLIFER & SEKHON, 1975). In the metathoracic legs of *Schistocerca gregaria* there is a single scoloparium containing about 24 cells (USHERWOOD et al., 1968); it is located closer to the distal end of the femur than the scoloparia in the other two pairs of legs. In the grasshoppers *Chortophaga viridifasciata* and *Romalea microptera*, MORAN et al. (1975) and SLIFER & SEKHON (1975) respectively have shown that the dendrites are paired in the scolopidia.

The TT organ of the cockroach has about 26 sensory cells and 14 scolopidia, and hence most of the dendrites are paired (YOUNG, 1970). In the TP organs of *Notonecta glauca* and the larva of *Anax imperator* there are even fewer sensory cells (WIESE & SCHMIDT, 1974, and this paper respectively). These are clearly separable into two scoloparia in *N. glauca*, a proximal one with three sensory cells and two scolopidia, and a distal one with five sensory cells and three scolopidia. Thus some dendrites are paired while others are single (WIESE & SCHMIDT, 1974). In *A. imperator* there is no obvious division of the cell bodies into two groups. Furthermore, only single dendrites have to date been observed in the TP organ of *A. imperator*. The TP organ thus has fewer sensory cells than any other arthropod joint chordotonal organ so far described.

In all insect limb chordotonal organs studied so far, the centriolar derivative is of type I (MOULINS, 1976), i.e. there is an axoneme from base to apex of the dendrite. In addition, all possess an apical, extracellular cap enclosing the distal end(s) of the dendrite(s).

In accord with other arthropod joint receptors, the sensory units in the TP organ of *A. imperator* are unidirectional (cf. e.g. YOUNG, 1970; MILL & LOWE, 1972; BOWERMAN, 1976; MILL, 1976; WRIGHT, 1976; MILL & HARRIS, 1977). There is some indication of position sensitivity of at least one unit and of variations in the adaption rates of different units, but further investigation is required to determine the detailed characteristics of the individual units.

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