Chordotonal Organs of Insects

Laurence H. Field\textsuperscript{a} and Thomas Matheson\textsuperscript{b}
\textsuperscript{a} Department of Zoology, University of Canterbury, PB 4800, Christchurch, New Zealand
\textsuperscript{b} Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK

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1. Introduction

Research on chordotonal organs has extended from the domain of sensory neurophysiologists to now include a diverse group of investigators in the areas of genetics, development, biochemistry, immunology, cytology, and evolution. Even within neurobiology, the demands for highly-specialised training and focus (e.g. motor control, transduction mechanisms, development or architecture of central circuitry) mean that many arthropod neurobiologists are not familiar with chordotonal organs. Comprehensive reviews of modern knowledge about chordotonal organs are lacking. Therefore it is our intention to provide not only a review of recent literature, but also to generate a practical guide for the diverse range of researchers mentioned above. We hope that by offering comments on fruitful and critical areas for research, we will encourage a cross-disciplinary approach in future research on chordotonal organs.

Chordotonal organs are found in the Insecta and Crustacea, but do not appear in any other arthropod classes. In insects, chordotonal organs occur in great morphological diversity (see
Section 3), and are found at nearly every exoskeletal joint, and between joints within limb and body segments. All chordotonal organs are internal mechanoreceptors and thus may serve as proprioceptors, or as highly specific mechanoreceptor organs (e.g. hearing organs). Morphologically, a chordotonal organ is a cluster of sensilla (rarely a single sensillum) connected to moveable parts of skeletal cuticle or to the tracheal system, or sometimes inserting into a connective tissue strand (Fig. 1a). Often, additional connective tissue elements anchor or support the organ. Within some chordotonal organs, neurons are clustered into one or two groups, termed *scoloparia*, which may be morphologically separated from each other (e.g. the mesothoracic femoral chordotonal organ of the orthopteran leg; Fig. 8d,e).

Chordotonal organs were first recognised in insects, and described by Graber (1882) based upon the presence of a terminal element (*Stifte*) in each sensory neurone, as viewed in the light microscope. This is a component of the *scolopidium*, the sensillum which characterizes chordotonal organs, and which is an elaborate micromechanical transducer. A scolopidium consists of one or more bipolar sensory neurons, each with a ciliated dendrite enveloped by two specialised cells, the *scolopale cell* and the *attachment (cap) cell*, plus a glial cell. The modified cilium forms a distal extension of each neuronal dendrite. The scolopale cell produces a barrel-shaped sleeve of fibrous *scolopale rods* (collectively termed the *scolopale*, or *Stifte* seen by Graber) which surround the cilium (or cilia) and insert distally into a dense *cap* produced by the attachment cell (Fig. 1b). The above terminology (reviewed by Howse, 1968) specifically applies to such internal sense organs and should not be applied to cuticular sensilla, as sometimes encountered in the literature (e.g. Dethier, 1963; Davies, 1988; Gillott, 1991; Wigglesworth, 1972). Chordotonal organs are not normally associated with external cuticular structures, such as hairs, bristles or campaniform sensilla, although evolutionary homologies to these are discussed in Section 12.

Other sense organs in insects (e.g. hair sensilla and campaniform sensilla) also contain bipolar sensory neurons with centriolar derivatives (“tubular bodies”) in the dendrites (Wright, 1976), but only chordotonal organ scolopidia contain the solid scolopale structure (scolopale rods). All mechanosensory sensilla with ciliated bipolar sensory neuron dendrites are together classified as Type I arthropod mechanoreceptor organs, whereas those mechanosensilla with multipolar, non-ciliated sensory neurons (e.g. joint and arthrodial membrane stretch receptors, and muscle receptor organs) are classified as Type II (Zawarzin, 1911).

The following terminology will assist in understanding the morphological variation found in insect chordotonal organs. Moulins (1976) described the various means by which the apical end of a chordotonal organ may be associated with the tegument. One of two basic types of structure are usually
Fig. 1. Chordotonal organ and scolopidium morphology, illustrated for the cockroach tarsal chordotonal organ. **a.** Diagrammatic illustration showing bipolar neurons with dendrites terminating in scolopidia embedded in connective tissue strands. The strands attach distally to the internal surface of the cuticle (not illustrated), and are stretched or relaxed during mechanical stimulation. **b.** Essential components of two scolopidia. In this case, each neuron gives rise to a ciliated dendritic outer segment which inserts along with the other from the paired neuron into the cap, and are surrounded by a scolopale produced by the scolopale cell. Attachment cells are embedded in strands. Neurons are surrounded by thick (left) or thin (right) glial cells (sheath and Schwann cells) with small nuclei. **a** and **b**, modified from Young (1970), with permission.
observed. To avoid confusion, we refer to either (a) non-connective chordotonal organs, or (b) connective chordotonal organs. In non-connective organs the scolopidia are connected distally to the hypodermis by the attachment cell (sometimes also an intermediate accessory cell); in connective chordotonal organs the scolopidia are inserted into a connective tissue strand via the attachment cell (the “connective” chordotonal organ of Howse, 1968). In the latter, the connective tissue strand often bridges a joint, as in some leg or antennal chordotonal organs. Although Moulins referred to this type as a “strand chordotonal organ”, we propose to use the terminology of Howse because a different kind of innervated connective tissue strand receptor (a multipolar Type II neurone) with a central cell body has subsequently been described in insect legs (“strand receptor” [SR] of Bräunig et al. 1981; Bräunig, 1985). Scolopidia that contain a single cilium and neuron are referred to as monodynal, whereas those with two or three cilia and neurons inserting into a common cap are referred to as heterodynal (Whitear, 1962). Invariably, heterodynal scolopidia have some ultrastructural difference between their sensory cells, the significance of which is unknown (Moulins, 1976).

Two distinct types of scolopodium are recognised: Type 1 and Type 2 (Moulins, 1976). They are distinguished by the nature of the cilium arising from the dendrite and their characteristics are summarised in Table 1, and described in greater detail in Section 4.

In Type 1 scolopidia, the cilium is of uniform diameter throughout and is anchored distally into an extracellular cap or tube that is secreted by the attachment cell. In Type 2 scolopidia the cilium expands distally, loses its axoneme and becomes a microtubule-rich cylinder which is loosely ensheathed in a dense tube (Moulins, 1976). In both types, the cap or tube is enclosed by the attachment cell, which makes connection to a source of mechanical displacement (Fig. 1).

Two further terms are often encountered in descriptions of chordotonal scolopidia. These refer to the morphology of the extracellular cap or tube into which the cilia insert. A mononematic scolopodium (Fig. 11a) contains a cap which is always subepidermal, whereas in an amphinematic scolopodium (Fig. 11b) there is no cap at the distal end of the cilium, but instead, the cilium is surrounded by a dense sheath-like tube which is drawn out into a thread that may insert into the cuticle or terminate subepidermally (Graber, 1882; McIver, 1985).

Following the classic reviews of Eggers (1928) and Debaisieux (1938), many authors have covered restricted aspects of chordotonal organ structure and distribution, often under the more general topic of arthropod mechanoreceptors or proprioceptors (Dethier, 1963; Bullock and Horridge, 1965; Finlayson, 1968; Howse, 1968; Rice, 1975; Wright, 1976; McIver, 1985). The past two decades have witnessed an expansion of knowledge about the roles of chordotonal organs in sensory feedback control of behaviour (earlier reviews by Rice, 1975; Wright, 1976 and Finlayson, 1976 in Mill, 1976), while recent reviews have dealt
Table 1. Characteristics of the two basic types of scolopidia, based upon ultrastructure (data from Moulins, 1976)

<table>
<thead>
<tr>
<th>Scolopidium type</th>
<th>Cilium characteristics</th>
<th>Terminal structure attachment</th>
<th>Distal cilium attachment</th>
<th>Proposed stimulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>a) Constant diameter</td>
<td>Extracellular cap (rarely tube)</td>
<td>Hemidesmosome anchors cilium to cap</td>
<td>Axial pull on scolopidium</td>
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<tr>
<td></td>
<td>b) Axoneme throughout</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>c) Distal dilation with microtubules</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 2</td>
<td>a) Enlarged distal segment beyond cilium</td>
<td>Elongate tube</td>
<td>Distal segment not anchored to tube</td>
<td>Lateral compression</td>
</tr>
<tr>
<td></td>
<td>b) Axoneme in cilium only, microtubules in enlarged distal segment</td>
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with increasingly narrower aspects of mechanoreceptor roles in insect behaviour (e.g. Bässler, 1993; Burrows, 1994). Others have reviewed chordotonal organ ultrastructure (Moulins, 1976), transduction mechanisms (French, 1988), homologies (Boyan, 1993) and the roles of tympanal organs in sound reception and behaviour (Michelsen and Larsen, 1985). In recent years new research areas, such as the genetic control of chordotonal organ development, have greatly increased our knowledge of chordotonal organs and their relationships to other sense organs (reviewed for Drosophila by Jan and Jan, 1993). It is timely that this diversity of information be gathered together and placed into an overall perspective through the present review. We build on the earlier general reviews of chordotonal organs rather than repeat the information contained therein.

At the time of the last major review on chordotonal organs (McIver, 1985) a number of aspects of the physiology of chordotonal organs remained unknown, although they had been posed as major research questions since the reviews by Wright (1976) and Moulins (1976). The foremost of these problems was the vast gap in knowledge about how a stimulus is applied to the dendrite. The question involves details about (a) the roles of scolopidial components in transferring mechanical distortion to the dendrite, (b) the functions of differentiated regions in the cilium, and (c) the differences in mode of sensory activation of the different scolopale types found in chordotonal organs. Initial progress was made on the role of the ciliary component (Moran et al. 1977). Another major question asked how different sensory responses are realised by adjacent chordotonal neurons, which often are clustered in a group, all receiving the same mechanical stimulus from a common cuticular termination. Differences must be sought in the electrical properties of the excitable membrane and associated transduction mechanisms, mechanical properties of the multicellular link between dendrite and stimulus, and ultrastructural or molecular differences between scolopidia. Two further questions remained: (1) what is the embryonic derivation and genetic control of chordotonal sensilla during development, and (2) what are the roles of chordotonal organs in the control of behaviour? McIver (1985) reviewed the scant literature on these subjects, but only in the past decade has a remarkable boom of research taken place to address these questions, and this forms an important part of the present review.

2. Histological methods for chordotonal organs in insects

2.1 Histochemical staining of fixed tissue

2.1.1 Baker-Masson's triple stain

Young (1970) fixed chordotonal organs in alcoholic Bouin's fluid, embedded them in paraffin and stained the 15 µm sections in Baker's modification of
Masson's triple stain. He reported that scolopale caps stained red and could be easily seen in the light microscope. This technique is useful for determining orientation of scolopales within a chordotonal organ.

2.2 INTRAVITAL PERFUSION TECHNIQUES

2.2.1 Methylene blue

The classical intravital methylene blue technique for invertebrate nervous system (Ehrlich, 1886) stains chordotonal neuron somata and axons, motor and CNS neuronal elements deep blue. Plotnikova and Nevmyvaka (1980) present a complete staining schedule. Owing to its ease of preparation, this technique is useful for temporary rapid surveys as well as for permanent staining of material for detailed wholemount study. Outstanding examples were illustrated by Zawarzin (1911).

2.2.2 Janus Green B

Brief 1-2 min application of a 0.02% solution of the vital stain Janus Green B to exposed tissue selectively stains the sheath of chordotonal organs and peripheral nerves while leaving other tissue transparent (Yack, 1993). The technique has been used successfully to locate fine nerve branches for physiological recording without apparent physiological detriment. It also enhances the contrast of scolopale caps against surrounding tissue to allow analysis of scolopale orientation, and stains chordotonal organ attachment strands and attachment cells in the crista acustica of orthopteran tympanal organs.

2.3 UPTAKE OF DYE BY CUT AXONS AND NERVES; INTRACELLULAR DYE INJECTION

2.3.1 Cobalt salts (acetate, chloride, hexammine, lysine) and nickel chloride

Since the introduction of the use of cobalt chloride in neuronal staining (Pitman et al. 1972), many authors have perfused cut sensory nerves with salts of cobalt or nickel to stain chordotonal neurons distally or their central projections proximally (e.g. Field and Pflüger, 1989; Masuko, 1989; Matheson and Field, 1990; Kent and Griffin, 1990; Mücke, 1991; Yack and Roots, 1992; Yack, 1992; Rössler, 1992a,b). It is also possible to record intracellularly from, and stain, individual chordotonal neurons using capillary microelectrodes filled with cobalt or nickel solutions (e.g. Delcomyn, 1981; Matheson, 1990) and to selectively identify neurons using a combination of both dyes in the same preparation (Sakai and Yamaguchi, 1983). Techniques are given in Strausfeld and Obermayer (1976); Tyrer and Altman (1974); Delcomyn (1981).
Methods of enhancing the precipitated cobalt ions are given in Tyrer et al. (1980) and Mesce et al. (1993).

2.3.2 Horseradish peroxidase

The enzyme horseradish peroxidase (HRP) has been used to fill cut axons to reveal projections of insect nerves. This technique is especially important since it allows viewing of filled cells in a transmission electron microscope. A complete review of the chemistry and technique is given by Nässel (1987).

2.3.3 Fluorescent dyes.

Since the introduction of the highly fluorescent dye Lucifer yellow (Stewart, 1978; reviewed by Strausfeld et al. 1983) for axon perfusion and intracellular filling of individual neurons, a number of additional dyes have become available (refer to Hau gland, 1994). These can be used to investigate sensory nerve central projections (e.g. Merritt and Murphey, 1992) or peripheral neuronal morphology. The carbocyanine dyes (e.g. Dil) may be applied directly to the cut ends of fixed or living sensory nerves, and will diffuse centrally or peripherally along chordotonal axonal membranes owing to their lipid solubility (Zill et al. 1993).

2.3.4 Neurobiotin

To date the technique of staining neurons with neurobiotin or biocytin (Horikawa and Armstrong, 1988) has not been applied to chordotonal sensilla, but when this is done the power of the method should yield new insights into, for example, the central projections of very small chordotonal sensilla such as those in the metathoracic FeCO, where cobalt staining has so far failed. Biotin has a low molecular weight and high affinity for avidin, which in turn can be conjugated with a range of histochemical markers such as fluorescein, rhodamine, Texas Red, Cy3 or horseradish peroxidase. Neurobiotin can be infused into the cut ends of nerves or injected through standard microelectrodes. Staining times are shorter than for cobalt, and microelectrodes are less prone to blockage. The tracer moves more rapidly and farther than cobalt. The range of conjugates available means that staining can be revealed either using fluorescent or standard light microscopy.

2.4 IMMUNOCHEMICAL TECHNIQUES

2.4.1 Antibodies against cell products

Anti-HRP antibody stains insect neuronal cell membranes and scolopales, and has been used to detect chordotonal organs in insect embryos (Jan and Jan,
Fig. 2. Labeling patterns of six antibodies that stain various components of cuticular sensilla and chordotonal scolopidia in *Drosophila*. 21A4 antibody marks the cytoplasm of all sensory neurons and a few central neurons. 49C4 antibody marks the cytoplasm of a subset of chordotonal organs only. Anti-HRP (horseradish peroxidase) antibody stains the cell surface of all sensory and central neurons. 21A6 antibody reveals only the terminal (tubular body?) of cuticular sensilla and the scolopales of chordotonal scolopidia, allowing a clear separation of the two types of sensilla. 58C12 antibody labels the cap and sheath cells of well-differentiated chordotonal organs in older (16-22h) *Drosophila* embryos. It is not clear whether the label is a cytoplasmic or a surface marker. 44C11 antibody labels the nuclei of all sensory and central neurons, allowing exact cell counts. Diagonal hatching indicates cytoplasmic or nuclear staining, solid lines indicate membrane staining, filled dots or scolopales indicate staining of accessory structures at dendrite tips and dashed lines indicate lack or staining with respective antibodies. Modified from Bodmer *et al.* (1987), with permission.
A glial-specific antibody (MAb 5B12; Meyer et al. 1987) preferentially labels glionexin, a glial protein in the extracellular matrix surrounding mechanoreceptors (including chordotonal organs) in crickets (Field et al. 1994). This promises to augment ultrastructural and physiological work on relationships between glial cells and chordotonal cells. Actin-rich scolopales (Wolfrum, 1990) can be labeled due to the specificity of rhodamine- or FITC-labeled phalloidin for actin. This technique was used in the investigation of chordotonal organ projections in embryonic Drosophila (Merritt et al. 1993).

Antibodies that label different parts of chordotonal and cuticular sensilla have been isolated from Drosophila (Bodmer et al. 1987). Fig. 2 compares labeling patterns for the two kinds of sensilla, and indicates how separate labeling of neuronal nuclei, plasma membrane, scolopale/cap cell and scolopale may be achieved. At least some of these antibodies (e.g. anti-HRP) label chordotonal organs in other insect genera, and could be useful tools for broader studies of insect sensory systems.

2.4.2 Antibodies against introduced molecules

Lucifer Yellow has been injected into embryonic chordotonal organ neurons and intensified with anti-Lucifer yellow antibody and diaminobenzidene (Merritt et al. 1993).

3. Diversity in distribution, structure and function

3.1 Overview of diversity

Chordotonal sensilla are utilised for a large variety of sensory functions in insects. In association with various internal tissues and cuticular structures, a chordotonal organ may function as a joint proprioceptor (connective chordotonal organ) in limbs, wings and the abdomen; a detector of substrate-borne
vibration (subgenual organ) or air-borne vibration (tympanal organ); a detector of antennal movement resulting from wind, gravitational forces or low frequency air-borne vibrations (Johnston's organ); or a monitor of cuticular distortion and bending in the wings and mouthparts (connective chordotonal organs) (Howse, 1968; Wales, 1976; Yack, 1992). In some of these sense organs the chordotonal sensillum may have exquisite sensitivity to movement, probably responding to displacements as tiny as 1 nm (French, 1988). The most comprehensive reviews of the distribution of chordotonal organs in the different insect families (Howse, 1968; McIver, 1985) often refer readers to the classical original surveys of Eggers (1928) and Debaisieux (1938), where the best illustrations of specific examples may be found.

The following is organised according to the anatomical location of chordotonal organs. For a cross-reference according to type of chordotonal organ, see Howse (1968).

3.2 Head

3.2.1 Mouthparts and antennae

Connective chordotonal organs occur in the mandibles, labium and maxilla (lacinia) of insects in the following orders: Orthoptera (grasshopper), Coleoptera (Bathysciinae, Elateridae), Diptera (mosquito), Homoptera (planthopper), Lepidoptera (butterfly) and Hymenoptera (termite) (Wales, 1976; McIver, 1985). It is probable that all insects possess these chordotonal organs in their mouthparts (McIver, 1985). Apical sensory organs (ASO, Lee and Altner, 1986) consist of scolopidia in the tips of labial and maxillary palps in larval stages of many insects (Zacharuk and Blue, 1971; Bloom et al. 1981), as well as in adults (Lee et al. 1988). In butterflies the ASO connects to the tip of the palp and does not monitor joint movement, but may detect mechanical deformation of the palp tip or internal haemolymph pressure changes (Lee et al. 1988). Because 1-3 of the sensory neurons undergo degeneration after other sense organs have differentiated in late larval development, the neurons of the ASO could serve as pioneer or guidepost cells for other axons growing towards the CNS (see Section 10.5). Masuko (1989) extended the above list with a description of a connective chordotonal organ in the honeybee maxillary palp (two scolopidia) and labial palp (12 scolopidia). These two organs do not cross joints and may monitor bending of the maxillary galea and the labial palp.

The antennal pedicel and scape each contain a connective chordotonal organ (Fig. 3a) in Orthoptera, Coleoptera, Hymenoptera, Diptera, Lepidoptera and Ephemeroptera, which spans the proximal joint of the segment (Eggers, 1928; McIver, 1985). The chordotonal organ in the pedicel (antennal connective chordotonal organ (Toh, 1981) or central organ (Schmidt, 1969)) is located centrally within a circular array of scolopidia belonging to the Johnston’s organ.
Fig. 3. Histology and external morphology of Johnston’s organ in insect antennae. a. Longitudinal section through the pedicel of the antennal base in *Chrysoperla* (Neuroptera). The small somata of Johnston’s organ neurons insert via amphinematic scolopidia distally into the joint at the articulation of the flagellum. The few larger somata of central organ (connective chordotonal organ) neurons insert onto the base of the flagellum with mononematic scolopidia containing caps. The antennal nerve is found medially. b. Johnston’s organ (arrow) appears as an inflated toroid at the base of the antennae in culicid and chironomid dipterans (shown is a male New Zealand mosquito, *Culex* sp.). a. Modified from Schmidt (1969), with permission; b. L.H. Field (unpublished), with permission.

Johnston’s organ occurs in nearly all orders of insects, including the Archeognatha and Thysanura, but reaches great elaboration in the Diptera (Culicidae, Chironomidae) where as many as 20,000 neurons innervate the organ and the pedicel is swollen to nearly half the diameter of the head (Fig. 3b) (Boo and Richards, 1975; Belton, 1989). McIver (1985) gives a complete
table of all studies on the structure of Johnston's organ up to 1985. Belton's (1989) scanning electron microscopic study provided a functional scheme for the operation of the system. Approximately 70 prongs radiate from a basal plate of the flagellum and become expanded into a system of pleats and grooves onto which attach the scolopidial sensilla. Flagellar movement deflects the prongs, causing the dendrites to be stretched while the scolopidial sheathing rods (tubes) remain firmly anchored.

Male mosquitoes and chironomid midges use Johnston's organ to detect conspecific female flight sound in mating behaviour (McIver, 1985). In honeybees Johnston's organ detects nearfield airborne sound generated by the wings at a frequency of 260 Hz during the waggle dance and used to convey information to other bees about sources of nectar in the environment (Dreller and Kirchner, 1993).

An unusual and phylogenetically important scolopidial organ is located in the distal (third) antennal segment of the collembolan *Allacma fusca* (Sminthuridae). Altner and Theis (1984) reported that two pegs have characteristics of both chemoreceptive hairs and chordotonal organs. The pore-bearing shafts are each innervated by four bipolar sensory neurons, while a fifth neuron inserts on the base of the peg via a ciliated dendritic outer segment enclosed in a scolopale. Its dendritic inner segment contains a prominent ciliary root of cross-banded material. Proximally the soma of the chordotonal-like neuron is attached either to antennal muscle or to a nerve moved by muscle. Crouau et al. (1987) describe a similar peg in another collembolan (Neanuridae). Ultrastructural evidence suggests that these scolopidial proprioceptors represent an intermediate stage between the mechanoreceptor sensilla attached to a moveable peg (hair) and the internal chordotonal organs which lack direct connections to external cuticular structures.

**3.3 THORAX**

Previous reviews have included only those thoracic chordotonal sensilla located in tympanal organs involved in sound reception. The review of Michelsen and Larsen (1985) is the best information source for morphology, biophysics and physiology of insect tympanal organs. We expand the list of thoracic chordotonal organs with two newly described types of thoracic ear and a complex chordotonal system in the ventral thorax which is partially associated with the coxal leg joint. In addition, recent work on moth wingbase chordotonal organs has shed light on their evolutionary origins.

**3.3.1 Thoracic tympanal organs**

Hearing organs in the thorax occur in the Hemiptera (e.g. water boatmen) and the Lepidoptera (noctuid moths) (Howse, 1968; Anderson, 1980). In both
groups a tympanal membrane on the thoracic wall or subcoxa is lined internally by an air-filled tracheal sac. Two or three scolopidia insert onto the inner surface of the tympanum (moths) or onto a club-like projection at the base of the tympanum (water boatmen) via a connective strand (Eggers, 1928; Ghiradella, 1971; Michelsen and Larsen, 1985).

A major new finding is the presence of a single thoracic cyclopean ear in the ventral midline of the thorax in preying mantids (Dictyptera). The external morphology is sexual dimorphic, but in both sexes a groove between the metathoracic legs leads to a deep cleft faced with two tympana (Yager and Hoy, 1986, 1987). Large air sacs line the tympana internally and a scoloparium of approximately 20 neurons lies in a ligament spanning from the tympanum to the ventral body wall. Some scolopidia are directed toward the tympanum while others are directed away from it. This unique feature has not been seen in any other insect auditory organ, and is one of several reasons for believing that the mantid ear evolved independently from other insect tympanal structures. Yager has since investigated 330 species of mantids, and has found a wide variation in complexity of cyclopean ears, including vestigial ones in atympanate mantids (Yager, 1989). The cyclopean ear is broadly sensitive to ultrasonic sound (25-45 kHz in Mantis religiosa), and is apparently adapted for detection of bat calls (Yager et al. 1990; Yager and May, 1990). Females can have reduced hearing sensitivity correlated with a reduction in tympanal structures (Yager, 1990).

A newly-discovered prosternal tympanal organ occurs in two genera of parasitic flies (Diptera: Tachinidae) (Lakes-Harlan and Heller, 1992; Robert et al. 1992, 1994). This organ consists of two medial, inflated prosternal tympanal membranes bound inside by a tracheal sac, the prosternal chamber (Fig. 4a,b,d). A pair of non-connective chordotonal organs arise from the frontal nerve and insert onto the cuticular tympanal membranes (Fig. 4c). Each scoloparium contains about 70 Type 1 neurons. Sexual dimorphism occurs, in that females have fourfold larger tympanal membranes than those of males, but smaller spiracles. This presumably reflects differences in acoustic behaviour between the sexes. Female tachinids are attracted to the calling song of male orthopterans (crickets and tettigoniids) and deposit maggots on the host (Walker, 1986). The frequency response of the tympanal organ matches the frequencies generated by the host. In the case of the tettigoniid host, this includes ultrasonic sensitivity (Lakes-Harlan and Heller, 1992).

3.3.2 Wings and wing articulations

The wing articulation region has a variety of mechanoreceptors, including a chordotonal organ at the wing base; sensory hairs and a chordotonal organ in the tegula; a multiterminal receptor (Type II); and campaniform sensilla on the wing veins (Howse, 1968; Orona and Agee, 1987). The best summaries of these receptors are given by Fudalewicz-Niemczyk and Rocsiszewska (1972),
Knyazeva (1988) and Fudalewicz-Niemczyk et al. (1993). Chordotonal organs of the non-connective type occur at the wing base and veins of wings of most insects, and in the halteres (modified wings) of dipterans (Howse, 1968). In lacewings (Neuroptera) a tympanal organ is located in the radius vein of the forewing and is thought to monitor ultrasound (Miller, 1970). The lacewing ear is unusual because it is fluid filled, rather than air filled as in nearly all other insect ears studied. Some lepidopterans have chordotonal organs associated with a thoracic tympanal membrane, in which case the structure acts as an ear (Treat, 1959; Roeder, 1966; Yack and Fullard, 1990).

The sensory structures in the locust tegula (a cupola-like structure at the anterior base of all four wings), include a non-connective chordotonal organ

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**Fig. 4. Anatomy of the prosternal ear of the tachinid fly Therobia leonidei.**

- **a.** Lateral view of a female, showing the inflated prosternal tympanal organ (arrow).
- **b.** Scanning electron micrograph of the ventral surface of the prothorax showing the inflated cervical membranes, which act as tympanal membranes (ty). The prothoracic coxa (co) lies to the left. The head has been removed leaving behind the neck (n).
- **c.** Enlarged lateral view of the tympanal organ, with the tympanal membrane partially cut away to reveal its internal anatomy. The chordotonal organ (arrow) extends along the ventral margin of the tympanal septum and is anchored to the sternal apophysis.
- **d.** Scanning electron micrograph of a ventral view of the chordotonal sensory organ (so) suspended within the tympanal cavity, which is lined by a tracheal sac. Scales, 100 µm. Modified from Lakes-Harlan and Heller (1992), with permission.
Fig. 5. Chordotonal organs associated with wings. a. Ultrastructure of the tegula of a locust wing. Longitudinal section showing representative chordotonal scolopidium inserting onto the hypodermis via an attachment cell. A single hair sensillum is also shown. b. Innervation and receptors associated with the wing of the African migratory locust. Note tegula at each wing base. Each wing chordotonal organ is associated with a multipolar stretch receptor (Type II) in a complex (arrows). Other multipolar and campaniform sensilla (solid ovals) are found on each wing. Branches of nerve 1 (n1) from the meso- and metathoracic ganglia are labeled. a, Modified from Kutsch et al. (1980); b, modified from Knyazeva (1987).

(Type 1) with about 30 scolopidia. Each scolopidium contains a single neuron (Fig. 5a). The chordotonal organ, and about 45 hair sensilla on the tegula monitor the downstroke part of the wing beat cycle (Kutsch et al. 1980).

Each wingbase of *Locusta* contains a chordotonal organ and multiterminal
stretch receptor (SR) complex (Fig. 5b) (Knyazeva, 1986). The mesothoracic wingbase chordotonal organ contains 25-27 scolopidia with distal attachments inserting onto the mesophragma (20 scolopidia) or into the connective tissue innervated by the single multiterminal receptor neuron near the postnotum (Gettrup, 1962; Altman and Tyrer, 1977a,b). The metathoracic wingbase chordotonal organ contains 19-20 scolopidia which are separated from the multiterminal receptor neuron and insert onto the pleurite. The wingbase chordotonal organ of the cricket contains 5 scolopidia and appears to be most sensitive to vibration, rather than to wing movement (Möss, 1971).

In the Diptera, the hind wings have been modified into small stalks, each with a knob at the tip, which are named halteres. They are articulated on the dorso-lateral metathorax and oscillate vertically in time with the wings (Pringle, 1957). At the base of each haltere is a large and a small non-connective chordotonal organ; the small one spans the haltere base transversely, whereas the large one inserts axially into the base. The large chordotonal organ contains 7-8 scolopidia in blowflies (Calliphora, Sarcophaga) which are all of Type 2, while the small chordotonal organ contains three scolopidia all of Type 1 (Pflugstädt, 1912). The proprioceptive role of halteres has been studied extensively since Pringle suggested their role as a gyroscopic stabilisation mechanism (Pringle, 1948; reviewed by Nalbach, 1993). The halteres detect angular velocity and acceleration generated by Coriolis force on the knob. Although the sensory receptors responsible for this sophisticated mechanism appear to be primarily the campaniform sensilla located in five fields at the haltere base (Hengstenberg, 1988), a role for the chordotonal organs has never been studied. Both the large chordotonal organ of the haltere, and Johnston's organ in the antenna are positioned to detect low frequency vibrations generated by wingbeats. Both organs have Type 2 scolopidial ultrastructure (tubular outer dendritic segment). Since Type 2 morphology is virtually restricted to these chordotonal organs, a study of their ultrastructure and physiology could elucidate the functional relationship between tubular scolopidia and sensitivity to low frequency mechanical vibration.

A wing base chordotonal organ and a multiterminal receptor cell also occur in the sphingid moth Manduca sexta, although the chordotonal organ contains only three Type 1 scolopidia (Yack, 1992). A hairplate receptor is also present at the wing base. Wing base sense organs have also been described in other moths, but they lack the full complement described above. For example, the noctuid moth Heliotis has the tegula receptors plus a “dense collection” of scolopidial sensilla in a non-connective chordotonal organ, but lacks the multiterminal receptor neuron (Orona and Agee, 1987). The atympanate saturniid moth Arctias has a chordotonal organ with three scolopidia, as in Manduca, but apparently lacks both the multiterminal neuron and the hair plate (Yack and Fullard, 1990; Yack and Roots, 1992). A strong case can be made for homology between the wing base chordotonal organ in the last two species and the tympanal chordotonal organs in tympanate moths (e.g. the
noctuid *Feltia*), as described in Section 12.3.2. Yack and Fullard (1993) cite eight lepidopteran families in which chordotonal organs appear to be associated with tympanal structures and are therefore likely to function as hearing organs, rather than as wing movement detectors.

Of 15 insect orders tabulated by Fudalewicz-Niemczyk *et al.* (1993), chordotonal organs occurred in *both pairs of wings and in the tegula* in Blattoidea, Mecoptera, Neuroptera, Lepidoptera and Diptera (forewings and halteres). Chordotonal organs occurred in *only forewings and tegula* in certain Orthoptera (crickets) and in Hymenoptera. Chordotonal organs occurred in *only the tegula* in other Orthoptera (grasshoppers and bush crickets), Isoptera and Plecoptera. No chordotonal organs occurred at the wing base region in the Dermaptera and Odonata. Another orthopteran suborder, Gryllacridoidea, has not been studied although it would be evolutionarily interesting inasmuch as it contains families with many primitive characters (e.g. Gryllacrididae, Stenopelmatidae, Haglidae). Fudalewicz-Niemczyk *et al.* (1993) conclude that the more primitive orders lack chordotonal organs in the proximal venation of the wings, while the higher orders do have chordotonal organs in this wing region. Often these orders include good flyers with less sclerotised, fairly long or wide wings with little venation. Exceptions to the general rule are the Gryllidae (Orthoptera), which have forewing chordotonal organs (these may be associated with the special function of the wings in producing sound), and some members of the primitive Blattoidea.

The positions of the wing base chordotonal organs suggest that they would be particularly well suited to detect deformation of the wings during folding, but whole-nerve recordings (Möss, 1971; Pearson *et al.* 1989) show that those of the cricket forewings and locust hindwings are mostly sensitive to vibration (and low frequency sound) and not to gross wing movement. Pearson *et al.* (1989) found that the afferents do not feed onto the flight motor neurones nor interneurones, but instead project to the auditory neuropile (mVAC). Therefore the chordotonal organs do not appear to contribute to flight pattern generation, but probably provide vibratory and low frequency acoustic input that could be used in orientation behaviour during flight.

### 3.3.3 Thoraco-coxal region

In locusts, the ventral region of the thorax contains a maximum of seven chordotonal organs per segment, some of which are associated with the coxa (Fig. 6b). The number and arrangement differ for the three thoracic segments (Table 2). The chordotonal organs fall into three groups: those connecting to sternites, those connecting to the coxa directly or indirectly, and those connecting to muscle (myochordotonal organs).

Those connecting to sternites and not associated with the leg joint include three in the prothorax (*anterior chordotonal organ* (aCO), *ventral chordotonal organ* (vCO), and *apodeme chordotonal organ* (apCO)), one in the mesothorax
Fig. 6. a. Innervation of a locust midleg (anterior view) revealed by CoCl₂ staining. (n5B1-4, main leg nerves; I-III, tarsal segments I-III. b. Schematic inner view of the right thoraco-coxal joint in a locust showing the positions of its proprioceptors. The dorso-ventral rotary axis of the three-axial joint traverses the condylus (C) of the joint and the proximal part of the coxa. (ajCO, anterior joint chordotonal organ; ajMS, anterior joint multipolar sensillum; COS, chordotonal organ strand system; jcMS, field of multipolar sensilla around the condylus; M92, anterior rotator muscle 92; pjCO, posterior joint chordotonal organ; pltnSR, pleuro-trochantinal strand receptor). a, modified from Mücke (1991); b, modified from Hustert (1983).
Table 2. Chordotonal organs of the locust whose projections are described in detail in Bräunig et al. (1981), Hustert (1978) and Pflüger et al. (1988).

<table>
<thead>
<tr>
<th>Body Region</th>
<th>aCO</th>
<th>cCO</th>
<th>vCO</th>
<th>apCO</th>
<th>ajCO</th>
<th>myoCO</th>
<th>FeCO (^a)</th>
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\(^a\)Both scoloparia stained together. \(^b\)Third abdominal segment.

References: \(^1\)Field and Pflüger (1989); \(^2\)Laurent (1987a); \(^3\)cockroach, Füller et al. (1981).
(apCO) and two in the metathorax (aCO, apCO). The aCO has diverged in function in the thoracic segments: in the prothorax it attaches to a cuticular plate in the middle of the cervical membrane and appears to comprise a rudimentary ear sensitive to sound and vibration of the cervical membrane (Pflüger and Field, pers. comm.); in the mesothorax it forms part of a web of four chordotonal organs which monitor coxal movement (Campbell, 1961; Hustert, 1978, 1982), and in the metathorax it connects to the meta-mesothoracic ventral suture. The vCO and the apCO attach to the basisternum and sternal apodeme, respectively (Bräunig et al. 1981). No functions have been demonstrated for the latter chordotonal organs, although they may be involved in moulting, regulation of internal pressure or ventilation (Bräunig et al. 1981).

The chordotonal organs associated with the coxa include the anterior joint chordotonal organ (ajCO, 26 neurons), the posterior joint chordotonal organ (pjCO, 6 neurons), and the coxal chordotonal organ (cCO, 15 neurons). In the prothorax the pjCO appears to be missing, but may be represented by a second scoloparium fused with the ajCO (Bräunig et al. 1981). In the metathorax the three chordotonal organs are present, while in the mesothorax an additional three non-joint chordotonal organs appear to have been recruited from the previously-mentioned sternite group to monitor coxal movement. The ajCO and pjCO are attached to the trochantin and posterior coxal articulating membrane, respectively, while the cCO is always attached to the coxal rim. In the mesothorax the cCO forms part of a cross-shaped system of interconnected strands from four chordotonal organs (COS; Fig. 6b) lying lateral to the mesothoracic ganglion. Of the four, only the cCO connects to the ventral rim of the coxa and therefore pulls on all of the interjoined COS ligaments during coxal movements. The other three include the aCO, with 11 neurons, the vCO with 11 neurons; and an additional posterior chordotonal organ, pCO, with 9 neurons. The elaborate investment of chordotonal organs and other mechanoreceptors in the mesothoracic coxal region suggests that the mesothoracic leg, rather than the pro- or metathoracic legs, plays a major role in peripheral sensory feedback control of walking. The chordotonal system responds phasically to rotary movements of the coxa and elicits reflexes in coxal motor neurons (Hustert, 1982, 1983; see Section 9.2.1).

The myochordotonal organ occurs only in the prothorax and metathorax. It attaches to neck muscle 57 in the prothorax and to a coxal rotator muscle, M121, in the metathorax (Bräunig et al. 1981, Bräunig, 1982b).

3.4 ABDOMEN

Previous reviews have cited the occurrence of abdominal chordotonal organs either as connective chordotonal organs in which an elastic connective tissue strand spans the abdominal segments (review by Finlayson, 1976), or as tympanal organs for sound reception in Orthoptera, Homoptera and Lepidoptera.
Yack and Fullard (1993) reviewed the occurrence of proposed (but not confirmed) hearing organs in the following additional insect orders: Blattaria (*Periplaneta*), Isoptera (*Zootermopsis*), Coleoptera (Cerambycidae, Cicindelidae, Dytiscidae) and Diptera (Sarcophagidae). Newly discovered chordotonal organs occur in the ovipositor, cerci and in the integument of the genital chamber.

*Connective chordotonal organs* are abundant on the abdominal pleura of larvae in Diptera, Lepidoptera, Coleoptera, Ephemeroptera, Odonata and Hymenoptera. In adults, the chordotonal organs are documented for Diptera, Coleoptera, Orthoptera, Phasmida, Blattaria and Hemiptera (Eggers, 1928; Finlayson, 1976). The development of pleural chordotonal organs has been well studied in Diptera (*Drosophila*) and Orthoptera (*Schistocerca*) (Campos-Ortega and Hartenstein, 1985; Meier and Reichert, 1990, Meier *et al.* 1991). In these insect orders, the elastic (connective) strand contains between one and seven scolopidia, and is located in one or more of the following constant positions: mid-ventral, ventro-lateral, lateral or dorsal. The sensilla respond to the compression phase of ventilation (expiration) in *Carausius morosus*, the only well-studied example (Orchard, 1975). In cicadas (Fig. 7a), there are three connective chordotonal organs associated with the tymbal (sound-producing apparatus): the tymbal, the tensor and the detensor chordotonal organs (Young, 1975; Doolan and Young, 1981). They exhibit a greater complexity of ultrastructure than the simple pleural chordotonal organs described above, and the number of scolopidia is much greater (maximal counts, respectively, are 1300, 350 and 400). Young (1975) compared the structure and number of scolopidia in cicada chordotonal organs with those of the proximal scoloparium of the femoral chordotonal organ in the orthopteran pro- and mesothoracic legs (see Section 3.5.3). In both, large numbers of tiny scolopidia are clustered at the proximal end to form an elongate capsule-shaped organ which attaches distally by a connective strand (Figs 7b, 8e). The demonstration that the proximal scoloparium is preferentially sensitive to vibration in the locust (Field and Pflüger, 1989) suggests that the two kinds of organ are similarly constructed for vibration reception. Details of structure/function relationships remain to be worked out.

Two newly discovered connective chordotonal organs occur in the distal part of the ovipositor of the cecidomyiid midge (Diptera) (Hallberg and Ahman, 1987). Each organ contains a single Type 1 scolopidium in an elastic strand which connects between the distal tip of the ovipositor and the more proximal ovipositor nerve. The arrangement suggests that the chordotonal organs respond to elongation of the ovipositor.

A single non-connective chordotonal organ (not reviewed previously) occurs at the base of each cercus in the cockroach (Dictyoptera) (Füller *et al.* 1981; Bernard *et al.* 1983). Its scoloparium contains 40-70 Type 1 neurons, which insert distally onto the anterior base of the cercus, and arise from nerve X of the sixth abdominal ganglion. This chordotonal organ inhibits cercal input.
Fig. 7. Abdominal chordotonal organs associated with the sound-producing tymbal in a cicada. 

a. Internal abdominal anatomy of *Cyclochila australasiae*, showing the right side of the abdomen viewed from the midline, with the large tymbal muscle removed. Three chordotonal organs (tymbal organ, tensor organ, detensor organ) are associated with the base of the tymbal and the tymbal muscle as well as the detensor tymbal muscle. In addition, the auditory chordotonal organ is stippled. 

b. Structure of the tymbal organ of another cicada, *Cystosoma saundersii*, which typifies the tightly-packed organisation of scolopidia with tiny neuronal somata in these chordotonal organs. Modified from Young (1975), with permission.
to three giant neurons in the sixth ganglion, and could potentially turn off the escape circuitry
during other behaviours (Goldstein and Camhi, 1988).

An important new discovery is the occurrence of individual scolopidia in the integument of the
genital chamber of the cricket *Teleogryllus commodus* (Sugawara, 1996). The scolopidia do not
form a chordotonal organ, but instead are distributed individually amongst the epidermal cells,
with the scolopale tips embedded in cuticle. All are amphinematic. About half have a single
Type 1 neurone (Table 1) while in the other half each has a Type 1 and a Type 2 neurone. The
solitary nature of the sensilla and details of their association with the cuticle have led Sugawara
(1996) to propose a new evolutionary scheme for derivation of chordotonal organ and cuticular
sensilla (see Section 12.6).

In grasshoppers (Acrididae) and moths (Pyralidae, Geometridae) tympanal organs occur in the
first abdominal segment: in uraniid moths and in cicadas, they are in the second abdominal
segment, while in axiid moths they are in the seventh segment (Howse, 1968). The morphology
is essentially similar to thoracic tympanal organs: a thin cuticular tympanic membrane on the
abdominal tergite forms the external surface of an internal hollow chamber lined by an air sac.
Often the tympanum lies within an external tympanic chamber that opens to the exterior via a
slit. The tympanal membrane is contacted by a connective chordotonal organ of variable
complexity and number of scolopidial neurons (1 to more than 1000, Yack and Fullard, 1990).
The variety and function of insect tympanal organs are reviewed by Michelsen and Larsen
(1985). Recently, Yack and Fullard (1990) have suggested that the A and B cells of the
tympanal organ of the noctuid moth *Actias luna* correspond to the three cells in the wing-hinge
chordotonal organ of an atympanate saturniid moth *Feltia heralis*, which primarily monitors
wing movements.

### 3.5 Legs

The greatest development in new knowledge of insect chordotonal organs has undoubtedly
taken place with the multitude of receptors in the legs. This can be attributed to the increase of
interest in the role of chordotonal organs in the
motor control of walking and in mechanisms of hearing in orthopterans. Earlier reviews cover
the following aspects of leg chordotonal organs: structure and distribution (McIver, 1985);
tympanal organs in insect legs (Michelsen and Larsen, 1985); tympanal organs in crickets (Ball
et al. 1989).

Chordotonal organs occur in every joint of the insect leg although the complexity is quite
variable. They fall into three classes: connective chordotonal organs which span joints and act
as proprioceptors; the non-connective feQoral chordotonal organ (FeCO), a proprioceptor that
is enlarged and elaborated in the Orthoptera; and the complex tibial organ, which in the
Orthoptera comprises a group of non-joint chordotonal organs with a complex arrangement.
It includes the subgenual organ (SGO), and the tympanal organ (composed of the crista acustica
and intermediate organ), all of which are sensitive to sound or vibration (or both) (McIver,
1985). Although original descriptions of the complex tibial organs were provided by
Schwabe (1906), those of Schnorbus (1971) for the cockroach tibia are more accessible and
useful for understanding the anatomy of the subgenual organ complex, as are those of Michel
(1974), Young and Ball (1974a), Ball and Young (1974) and Lakes and Schikorski (1990) for
the tympanal organ. Previous reviews discussed subgenual organs in the Orthoptera, Blattaria,
Hymenoptera, Isoptera and Lepidoptera (Debaisieux, 1935, 1938; McIver, 1985). Here we
cover newly discovered chordotonal organs in legs of other orders, and discuss the detailed
knowledge that has developed for the orthopteran FeCO.

3.5.1 Innervation atlas

Grosch et al. (1985) and Mücke (1991) used cobalt infusion (15% CoCl₂ in distilled H₂O) of
cut leg nerves to fill and map the entire sensory complement of the locust mesothoracic leg
nerves distal to the trochanter (Fig. 6a). In the moth Manduca sexta, Kent and Griffin (1990)
used cobalt axonal infusion to describe sensory innervation of the entire leg. Similar maps have
been published earlier for cockroach (Nijenhuis and Dresden, 1952); cricket, (Fudalewicz-
Niemezryk et al. 1980); and termites (Richard, 1950). Hustert et al. (1981) filled nerves to the
thoraco-coxal region in the locust leg (the thoracic chordotonal organs have been described in
Section 3.3). The resulting atlases are useful for locating the positions of leg chordotonal
organs, multiterminal proprioceptors and external hair sensilla, mapped according to nerve
supply from the CNS (Mücke, 1991). Such information is potentially valuable to developmental
biologists and geneticists working on the sensory nervous system of insects. In the general case,
represented by the Orthoptera, three chordotonal organs within the leg proper are associated
with joints, and two (three if the prothoracic leg tympanal organ is present) are not associated
with joints but function as sound or vibration detectors in the tibia. The chordotonal organs
associated with the coxa occur in the thorax, and not the leg proper (Section 3.3.3).
3.5.2 Coxal and trochanteral chordotonal organs.

No chordotonal organs have been found within the coxa or trochanter of any of the three pairs of legs in the locust. The fusion of the trochanter to the femur may explain the lack of chordotonal organs in the trochanter, but not their lack in the coxa. Instead, a single multiterminal strand receptor (Type II mechanoreceptor) in the coxa monitors the trochanter’s movement (Bräunig et al. 1981). It is not known what determines the occurrence of Type I or Type II receptors in joints.

3.5.3 Femoral chordotonal organ

The femur contains the (non-connective type) femoro-tibial chordotonal organ (FeCO), which is anchored proximally to the dorsal anterior hypodermis of the femur and stretched distally by a thin cuticular apodeme inserted onto the tibia, beside the extensor tibiae muscle apodeme. The FeCO probably occurs in all orders of insects (Debaisieux, 1938; Howse, 1968), but details have been reviewed for the Orthoptera. Recent studies have added descriptions of the FeCO in Lepidoptera, Neuroptera, Hemiptera and Diptera. In Manduca sexta (Lepidoptera), the FeCO consists of a scoloparium containing a “large cluster” of neurons located near the trochanteral-femur joint (Kent and Griffin, 1990). This attaches to a long apodeme extending to the tibia, which is the common pattern seen in Orthoptera. Devetak and Pabst (1994) reported the presence of an FeCO in the legs of the lacewing Chrysoperla carnea, but did not give details of morphology. In the hemipteran bug Nezara viridula, the FeCO scoloparia are anchored to the anterior face of the distal third of the femur and lacks the distal apodeme. Instead they attach directly to the tibial extensor muscle and muscle apodeme by three connective tissue strands which extend from the FeCO (Michel et al. 1983). There are 12 scolopidia of Type 1, each containing two dendrites. The scolopidia are grouped into three scoloparia: two are in the form of capsules attached to the femur wall and the third consists of four scolopidia distributed along the strand that attaches to the extensor apodeme.

The Diptera appear to have two separate FeCOs, but they have a common single origin. The FeCOs comprise three scoloparia. In the blowfly Phormia, one of the scoloparia is located distally in the femur, near the site of embryonic origin, while the other two reside near the trochanter border, to where they migrate during development (Lakes and Pollack, 1990). A recent description of the FeCO in the legs of Drosophila indicates that this arrangement does not exist in the Drosophilidae (Shanbhag et al. 1992). Some 74 scolopidia are organised into three scoloparia anchored proximally. There is no FeCO apodeme (similar to the morphology of the hemipteran FeCO above). Instead, two scoloparia (14 and 25-28 scolopidia) insert distally onto femoral muscle fibres via elongate attachment cell strands, and the third (42 scolopidia)
attaches to the distal rim of the femur. This means that the FeCO is not directly stretched by connection to the tibia, but instead must be stimulated indirectly by relative displacement of the muscles in the femur.

A most unusual peripheral “glomerulus” of synaptic endings of FeCO afferents is found in the FeCO nerve in the proximal femur of Drosophila melanogaster (Shanbhag et al. 1992). No such peripheral synaptic structures have ever been described for chordotonal organs in other insects, although Foelix (1975) demonstrated such structures in arachnids. In Drosophila, TEM evidence reveals chemical synapses of convergent, divergent, reciprocal and serial types. This suggests that a significant amount of peripheral information processing must be occurring before the FeCO afference reaches the thoracic ganglia in flies. The extent of peripheral processing in arthropod mechanosensory integration merits further investigation, since peripheral synaptic interactions between other afferents are well-known elsewhere in arthropods (e.g. lateral inhibition in the compound eye; Bullock and Horridge, 1965).

Lepidopteran, hemipteran and dipteran FeCOs are simpler than those in the Orthoptera, both in structure and numbers of scolopidia. The FeCOs in the locust have received the greatest attention (Burns, 1974; Field and Pflüger, 1989; Matheson and Field, 1990). All femoral chordotonal organs investigated to date contain Type 1 scolopidia, each containing two neurons (McIver, 1985). The pro- and mesothoracic FeCOs are divided into two tear-shaped scoloparia anchored in the proximal femur and attached to a long needle-like cuticular apodeme which extends the length of the femur to the tibia (Fig. 8a,e). The proximal scoloparium contains several hundred very small neurons and is more sensitive to vibrations than the 42 larger neurons of the distal scoloparium, which is sensitive to movement and displacement of the tibia. The distal scoloparium mediates postural resistance reflexes of the tibia, whereas the proximal scoloparium causes weak excitatory reflexes that are direction
Fig. 8. Variation in femoral chordotonal organs of orthopterans, according to leg position and species. 

a. The FeCO of locust pro- and mesothoracic legs consists of two separate scoloparia in the proximal part of the femur both of which attach to a common apodeme running the length of the femur.

b. In the locust metathoracic leg the FeCO consists of a single organ attached to a short apodeme in the distal part of the femur.

c-e. FeCO scoloparia range from fully-merged (locust metathoracic FeCO (c), partially fused (New Zealand weta (Stenopelmatidae) metathoracic FeCO (d), to fully separate (pro- and mesothoracic FeCO of all orthopterans examined (e). Neurons of the proximal scoloparium in the pro- and mesothoracic FeCO are much smaller than those in the distal scoloparium. A similar size distinction in the locust metathoracic FeCO suggests that two scoloparia are also present here. Scale c-e: 500 µm. 

a, modified from Field and Pflüger (1989), with permission; b, L. H. Field (unpublished), with permission; c-e, modified from Matheson and Field (1990), with permission.
Insensitive (Field and Pflüger, 1989). This was the first demonstration of a functional difference between scoloparia of a chordotonal organ; the same functional difference was subsequently found in the FeCO of the stick insect (Kittmann and Schmitz, 1992).

In ensiferan orthopterans (Stenopelmatidae: Field and Rind, 1976; Tettigoniidae: Theophilidis, 1986a; Gryllidae: Nowel et al. 1995) the pro- and mesothoracic FeCOs are not different from that in the metathoracic leg. However in the caeliferan orthopterans (locusts, grasshoppers), the metathoracic FeCO is markedly different in location, morphology and cell number to those of the more anterior legs (Fig. 8a,b). When different orthopteran FeCOs are compared, a variable degree of fusion of scoloparia is found (Fig. 8c-e). Complete fusion is seen in the caeliferan metathoracic FeCO (Fig. 8c), partial fusion is seen in the ensiferan FeCOs (Fig. 8d) while unfused scoloparia are seen in caeliferan pro- and mesothoracic FeCOs (Fig. 8e).

The locust metathoracic FeCO has recently been shown to be much more complex than previously thought. Rather than containing 24 to 50 neurons (as reported by Usherwood et al. 1968 and Zill, 1985a) it contains an average of 92; the increase being entirely due to a population of tiny neurons that had been overlooked earlier (Matheson and Field, 1990). These cells appear to be homologous to the proximal scoloparium of the pro- and mesothoracic chordotonal organs. The metathoracic chordotonal organ is anchored distally and therefore has a shorter apodeme than those of the pro- and mesothoracic organs. The structure and arrangement of the viscoelastic ligament joining the FeCO to the apodeme is complex. It creates a mechanical basis for range fractionation of the neuronal response to movement and position of the tibia (Field, 1991). The ligament is composed of two parallel bundles of strands: the first one (dorsal ligament) appears relatively inelastic, is fused, and inserts onto the proximal tip of the apodeme, whereas the second one (ventral ligament) is relatively elastic and its strands insert sequentially along the apodeme (confirmed by Shelton et al. 1992). As the tibia is flexed and the FeCO is stretched, a sequential recruitment of tension occurs, first onto the distal elastic strands then progressively onto the more proximal elastic strands and finally onto the inelastic bundle. Neuronal responses reflect the recruitment as larger spikes and higher tonic firing frequencies appear progressively during extension.

Theophilidis (1986a) showed a further elaboration in the apodeme/ligament of the metathoracic FeCO in the bush cricket Decticus albrifrons. There the two scoloparia are nearly fused but the elastic ligaments are still separated until they insert onto the common cuticular apodeme, resembling the morphology seen in Fig. 8b for the New Zealand weta. The apodeme of the proximal scoloparium (and not the distal one) extends into the ligament as a thin cuticular tube which looks like a “zig-zag” string running inside the elastic tissue. Stretch causes this folded cuticular strand to progressively unfold proximally (cricket metathoracic FeCO: Nowel et al. 1995). Presumably the mechanism
is analogous to that of the locust and causes progressive recruitment of neurons.

The functions of the metathoracic FeCO include control of postural resistance reflexes of the tibial flexor and extensor muscles (see Section 9.1), as well as reflex feedback onto muscles in other leg joints (Field and Rind, 1981). In stick insects, the FeCO also mediates joint control in cataleptic behaviour (reviewed by Bässler, 1993).

The theme of complexity in the metathoracic FeCO continues with the discovery of two associated sensory structures. The ventral “flexor strand” (Field and Burrows, 1982) is a highly elastic strand which links the FeCO to the flexor tibiae muscle apodeme. It contains the dendrites of a single stretch-sensitive strand receptor neuron (SR) which belongs to a unique class of insect mechanoreceptors with somata in the CNS (Bräunig, 1982a, 1985). Its axon passes across the FeCO but continues separately to the CNS, joining nerve 3 in the coxa, rather than joining the axons of the FeCO in nerve 5B1 (terminology: Campbell, 1961). The second associated receptor is a multiterminal receptor neuron which branches from nerve 5 at the base of the FeCO to innervate the accessory flexor muscle (Matheson and Field, 1995).

3.5.4 Tibial subgenual, tympanal and tracheal organs

In many orders a subgenual organ (SGO) occurs in the proximal part of the tibia of all legs. This often consists of a septum which partially occludes the haemocoel, and detects substrate vibration in orthopterans and hemipterans; and sound as well as substrate vibrations in the cockroach *Periplaneta* (Shaw, 1994a,b). A second chordotonal organ (the tracheal organ) occurs adjacent to the SGO, but lies axially along the anterior tibial trachea. In the ensiferan Orthoptera, however, each prothoracic leg often possesses an external cuticular modification, comprising an ear with paired tympanal membranes, on the tibia immediately distal to the SGO. In such cases the tracheal organ has become elaborated into a tympanal chordotonal organ concerned with hearing, and this, together with the SGO, is termed the complex tibial organ. Details of the physiology of these chordotonal organs has emerged in a few species, especially with recent interest in studying vibrational communication amongst insects. We first review the basic plan and recent developments in knowledge of the orthopteran SGO, and then proceed to the tympanal organ and tracheal organs in various groups.

3.5.4.1 Subgenual organs The basic structure of the SGO is seen in Fig. 9c (Orthoptera) and Fig. 10a (Blattaria). The scolopidia occur around the periphery of a fan-shaped septum oriented at right angles to the leg axis and marked externally by a group of campaniform sensilla. In the cockroach (Fig. 10a), nerve 5B1 enters the tibia on the anterior side and immediately bifurcates to supply an array of 22-26 scolopidia around the periphery of the
Fig. 9. Chordotonal organs in the proximal tibia and the tarsus. 

a. Tracheal organ in the left mesothoracic leg of a cricket, viewed dorsally. Attachment cells omitted. The scolopidia lie dorsally on the tracheal branch and a connective tissue membrane spanning between this branch and the tracheal trunk. 

b. Tarso-pretarsal chordotonal organ in the backswimmer *Notonecta*. A distal scoloparium of five neurons attaches to the claws while a proximal scoloparium with three neurons inserts onto the unguistractor plate. 

c. The complex tibial organ in the prothoracic leg of the New Zealand weta *Hemideina* (Stenopelmatidae) is similar to that of bush crickets (Tettigoniidae), but differs from that in the crickets (Gryllidae). The fan-shaped subgenual organ arises from a pocket in proximal inflated tracheal chamber and is supplied by branches from the tympanal nerve and a branch from the main leg nerve N5. A small collection of scolopidia forms the accessory organ at the base of the fan. The intermediate organ and crista acustica arise from the tympanal nerve and lie atop the anterior tympanal vesicle, which is apposed to the tympanal membrane of the eardrum. 

d. The complex tibial organ of the cricket (*Gryllus*) prothoracic leg, viewed from anterior. a, modified from Young and Ball (1974b), with permission; b, modified from Wiese and Schmidt (1974), with permission; c, modified from Ball and Field (1981), with permission; d, modified from Michel (1974), with permission.
fan. At the base of the fan, which inserts onto the posterior wall of the tibia, is a group of 10 scolopidia (the *nebenorgan*; Schnorbus, 1971). On the anterior side of the fan another separated cluster of 10 scolopidia (the *distal organ*) receives innervation from a more distal branch of N5B1 and inserts distally onto the anterior trachea.

Complete descriptions of orthopteran SGOs are found in Schumacher (1979) for Tettigoniidae (bush crickets); Young and Ball (1974a) for Gryllidae (crickets); Ball and Field (1981) for Stenopelmatidae (wetas). They differ from that of the cockroach in that the SGO is innervated partially by nerve 5B1 and partially by a sensory branch of the main leg nerve, N5B2 (Fig. 9c). They also have more neurons (18-25 for crickets, 50-63 for bush crickets and wetas; Ball and Field, 1981). All the scolopidia described so far are of Type 1. A newly-described SGO in the tettigoniid *Phasmodes ranatirformes* (stick insect mimic) consists of a supporting membrane that hangs in the haemolymph channel, with 18-23 scolopidia lying in parallel on its periphery, beneath the dorsal anterior cuticle of the tibia. The tympanal nerve innervates these cells as a single array, rather than from two branches entering from anterior and posterior sides of the proximal tibia as seen in other tettigoniids, crickets and wetas (Lakes-Harlan *et al.* 1991). Other recent tettigoniid descriptions have been given for a Chinese bush cricket *Gampsocleis gratiosa* (33 Type 1 scolopidia, each with a single dendrite (Lin *et al.* 1993), and the European bush cricket *Ephippiger ephippiger* (22-24 scolopidia). Both sense organs differ little from descriptions for most other tettigoniids (e.g. Schumacher, 1975).

In other orders (reviewed in McIver, 1985) the SGO is club-shaped (Hymenoptera), cup-shaped (Isoptera), or diffuse and unattached distally.
Fig. 10. Subgenual organs in non-orthopteroid groups. **a.** Dorsal view of the subgenual organ complex in the left leg of the cockroach *Periplaneta*. Neuron somata are shown for the dorsal organ, the subgenual organ and the neben (side) organ, as well as for campaniform sensilla. **b.** Subgenual organ in the left mesothoracic tibia of the lacewing *Chrysoperla* (Neuroptera). Three scolopidia (neurons labeled nS1-3) insert onto a lens-like velum comprising modified attachment cells of the scolopidia. **c.** Schematic longitudinal section showing the ultrastructure of one of these scolopidia. a, modified from Schnorbus (1971), with permission; b,c, modified from Devetak and Pabst (1994), with permission.
(Lepidoptera), and apparently lacking in Coleoptera and Diptera (Howse, 1968; McIver, 1985); homologues of the SGO should be sought in the last two orders. In the tibia of the lacewing Chrysoperla carnea (Neuroptera) a highly simplified SGO occurs with three Type 1 scolopidia, each with one dendrite (Devetak and Pabst, 1994). The capsule-like scoloparium is attached to the proximal dorsal wall of the femur and gives off three thin strands (attachment cells) distally which enlarge and flatten out into a transverse lens-shaped “velum” which occludes the haemolymph channel by spanning from the tibial wall to the laterally appressed trachea (Fig. 10b,c). The organ apparently detects substrate vibrations used in intraspecific communication (Devetak, 1992). In the Lepidoptera (Manduca sexta) a subgenual organ consisting of 30 neurons occurs on the dorsal wall of the mid-tibia of pro- and mesothoracic legs, but they are not obviously attached to any fan-shaped membrane traversing the tibial lumen (Kent and Griffin, 1990). The ultrastructural characteristics of the scolopidia are not known.

A highly unusual variation on the mechanism of suspension of the SGO occurs in the hemipteran bug Nezara viridula (Michel et al. 1983). This simplified organ consists of a capsule-like scoloparium of two scolopidia branching off the tibial nerve, and anchoring proximally to the dorsal tibial wall. The scolopidia give rise distally to a broad flat ligament (two attachment cells from the scolopidia) resembling a banner, and suspended longitudinally in the haemolymph channel of the tibia. This differs from all previous SGO descriptions in that the structure runs lengthwise along the haemolymph channel rather than transversely. Distally the ligament forks into two tapered strands, which loosely connect to the two nerves at the distal end of the tibia. The ligament appears to be essentially free to bend or vibrate in the channel, and therefore is likely to be stimulated in a different manner from that expected in other SGOs, where fluid acceleration against the transverse septum in thought to be the effective stimulus (Howse, 1968; Schnorbus, 1971).

3.5.4.2 Tympanal organs in ensiferan orthopterans In the ear of the crickets (Grylloidea), the scolopidial tympanal organ of the prothoracic leg is attached to an enlarged internal trachea lying immediately beneath one (or two) oval,
cuticular tympanal membrane(s) on the anterior (and posterior) faces of the proximal tibia (Fig. 9d). The scolopidia are distributed into three groups, but the composition, and innervation from the tympanal nerve, varies amongst genera and species and whether the scolopidia are anchored to the tegument by primary attachment cells only, or by primary and secondary attachment cells (Ball et al. 1989). In the meso- and metathoracic legs the homologous array of scolopidial cells (tracheal organ, Fig. 9a) is not associated with a tympanal membrane (Young and Ball, 1974b). The structure, development and physiology of the tympanal organs in crickets have been reviewed by Ball et al. (1989).

3.5.4.3 The tympanal organ-SGO complex in bush crickets or katydids (Tettigonoidea), wetas and king crickets (Gryllacridoidea), is more elaborate (Fig. 9c) and the tibia is highly modified for sound reception. Both tympanal tracheae are swollen to occupy much of the internal width of the tibia at the level of the tympanal membranes. The tympanal organ consists of a large series of scolopidia, the *crista acustica*, with neurones evenly graded in size distally (Schwabe, 1906). The *crista acustica* of the tettigoniids corresponds to the distal group of scolopidia in the cricket (Young and Ball, 1974a). An additional small group of scolopidia, the *intermediate organ* (*Zwischenorgan* of Schwabe, 1906), lies between the SGO and the *crista acustica* (and appears homologous to the “proximal group A” cells in the cricket (Table 3). A comparison of the scolopidial composition of complex tibial organs in gryllids, tettigoniids and stenopelmatids (Table 3) showed that larger numbers of scolopidia occur in the SGO and intermediate organ of the last two families, compared to counts in gryllids, but that numbers were similar for the tympanal organ and *crista acustica* of all families (Ball and Field, 1981). This presumably relates to different requirements for substrate vibration sensitivity, but not for frequency sensitivity or discrimination.

Typically, the scolopidial neurons of the *crista acustica* lie in an axial row along the dorsal surface of the anterior trachea. The sensilla are oriented perpendicular to the leg axis and suspended in a fluid-filled tent which is anchored via attachment cells to the dorsal tegument of the tibia (Fig. 9c) (Howse, 1968; Young and Ball, 1974a). The sensilla of the intermediate organ lie along the trachea, rather than at right angles to it as in the *crista acustica*.

In an unusual Australian tettigoniid (*Phasmodes ranatriformes*), which mimics stick insects, the prothoracic leg lacks an auditory tympanum, yet the internal complex tibial organ contains a *crista acustica*, SGO and intermediate organ (Lakes-Harlan et al. 1991). The mimic is totally deaf to airborne sound, even though the vibrational sensitivity is comparable to that of other tettigoniids. The *crista acustica* contains 16-18 neurons in the same ordered arrangement found in tympanate tettigoniids and apparently represents a primitive form in the evolution of the auditory system in tettigoniids.

Two major differences occur in a primitive ensiferan, the New Zealand weta
Table 3. Terminology and number of scolopidia in complex tibial scolopidial organs of orthopteran insects (modified from Ball and Field, 1981).

<table>
<thead>
<tr>
<th>Family and species</th>
<th>Authority</th>
<th>Terminology and number of scolopidia</th>
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<tr>
<td><strong>Gryllidae</strong></td>
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<td></td>
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<tr>
<td><em>Gryllus campestris</em></td>
<td>Eibl, 1978</td>
<td>Subgenual (25) Frontal process (8-10) Caudal process (10-12) Main process (35)</td>
</tr>
<tr>
<td><em>Gryllus bimaculatus</em></td>
<td>Michel, 1974</td>
<td>Subgenual Proximaler Abschnitt (10-12) Hinterer Tympanalnerv (10-12) Vordere Gruppe (35-40)</td>
</tr>
<tr>
<td><em>Teleogryllus commodus</em></td>
<td>Young and Ball, 1974a</td>
<td>Subgenual Proximal group A (7-9) (20) (40-43)</td>
</tr>
<tr>
<td><strong>Tettigoniidae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Tettigonia viridissima</em></td>
<td>Schwabe, 1906</td>
<td>Subgenual Zwischen-organ (63) (17) Crista acustica (40)</td>
</tr>
<tr>
<td><strong>Stenopelmatidae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Hemideina crassidens</em></td>
<td>Ball and Field, 1981</td>
<td>Subgenual Intermediate organ (50) (19) Crista acustica (46-57)</td>
</tr>
</tbody>
</table>

* These scolopidia were grouped differently by Young and Ball (1974a) and therefore the groupings are not named here.
(Stenopelmatidae): the tent enclosing the scolopidia is filled with an ordered, gelatinous mass of attachment cells which are anchored to the dorsal tegument, and the tympanal membrane is unusually thick (60-110 µm). In all other respects the anatomy of the weta tympanal organ conforms to that observed in the Tettigoniidae rather than the Gryllidae (Ball and Field, 1981).

In the orthopteran crista acustica, the graded size of sensory neurons from proximal to distal (Fig. 9c) corresponds with a tonotopic sensitivity of the individual neurons, whereby the proximal, larger cells are tuned to low frequencies and the distal small cells are tuned to high frequencies (Oldfield, 1982; Lin et al. 1993). This tonotopic sensitivity is maintained during postembryonic development, despite enlargement of the tympanal organ in successive instars. In the tettigoniid Ephippiger ephippiger, tuning sensitivity curves from the 4th instar onwards resemble the adult tuning curves even though the morphologies of the tympanal membrane, acoustic trachea and tympanal covers change developmentally (Rössler, 1992b). The solution to the problem of maintaining consistent tuning curves lies in retention of a relatively constant cap cell width and constant areas of tectorial membrane and dorsal wall which contact the crista acustica, through the instars. However, the hearing sensitivity of instars 4 through 6 is much less than that of the adult (20-30 dB difference), and only increases after moulting from instar 6 to adult. This corresponds to a dramatic increase in diameter of the prothoracic spiracle with that moult (Rössler, 1992b).

All scolopidia of tibial tympanal organs described to date are of Type 1 structure with a single mononematic dendrite (Michel, 1974; Ball, 1981; Houtermans and Schumacher, 1974; Lin et al. 1993).

The tracheal organ in the meso- and metathoracic legs has been studied in the cricket Teleogryllus commodus (Young and Ball, 1974b) and the tettigoniid Ephippiger ephippiger (Rössler, 1992a,b). In the cricket, it has fewer scolopidia than in the prothoracic leg (average of 12 scolopidia versus 40-43, respectively), and appears to represent only the two proximal groups of scolopidia found in the prothoracic leg. The entire distal group of an additional 27-29 scolopidia is absent.

In the tettigoniid, the tracheal organs of the meso- and metathoracic legs (named cristae acusticae by Rössler, 1992a) also differ significantly from the crista acustica of the prothoracic leg. The meso- and metathoracic tracheal organs are not associated with tympana, enlarged tympanal cavities nor with enlarged spiracular openings, as found in the prothoracic condition (all are adaptations for sound conduction and enhancement). The numbers of scolopidia are reduced: prothoracic crista acustica, 28; mesothoracic tracheal organ, 11; metathoracic tracheal organ, 7. The scolopidial structure in the meso- and metathoracic tracheal organs differs from that in the prothoracic
crista acustica: in the former, (a) the caps are only loosely applied to the tectorial membrane, and (b) the scolopales are significantly smaller. These micromechanical differences probably restrict the sensitivity of the meso- and metathoracic tracheal organs to low frequency sound or vibration (Rössler, 1992a); in contrast, the prothoracic crista acustica is specialised to detect high frequency sound, and is apparently isolated from vibratory energy (Shaw, 1994a). It is likely that the meso- and metathoracic tracheal organs represent the evolutionarily primitive condition of these sense organs, and that the prothoracic crista acustica represents an apomorphic specialisation.

3.5.5 Tibio-tarsal and tarso-pretarsal chordotonal organs

Chordotonal organs spanning the tibio-tarsal joint are of the connective type. The best studied, that of the cockroach, *Periplaneta americana*, contains 26 mononematic neurons grouped into three categories (thick dendritic sheath, heterodynam; thin dendritic sheath, heterodynam; thin sheath, monodynam) (Fig. 1b). The connective strand forms three branches, each with scolopidia that respond unidirectionally to deflection of the tarsus (Young, 1970) (Fig. 1a). The tibio-tarsal chordotonal organ in the locust *Schistocerca gregaria* (Fig. 6a) contains about 30 neurons (maximum 34, Laurent, 1987c) which decrease in size distally, in two scoloparia (Kendall, 1970; Mücke, 1991).

The tarso-pretarsal chordotonal organ in the tarsus has been described for Hemiptera, Lepidoptera, Diptera and Orthoptera. The hemipteran organ (backswimmer, Notonectidae; pond skater, Gerridae; reviewed by McIver, 1985) is divided into two spindle shaped scoloparia, the more proximal of which contains three scolopidia, and the distal of which contains five scolopidia inserting onto the unguitractor plate of the claws (Fig. 9b) (Wiese, 1972; Wiese and Schmidt, 1974). A similar chordotonal organ, recently described in the lepidopteran *Tineola biselliella* (Tineidae), is of the non-connective type, with two scoloparia (Fauchuex, 1985). The more proximal scoloparium (attachment not clear) has three amphinematic scolopidia (1-3 neurons each). The more distal scoloparium has one mononematic scolopidium with three neurons, and attaches to a cuticular invagination at the pretarsal articulation via a short attachment cell. This particular organ presents an excellent possibility for neurophysiologists to study the relationship of ultrastructure to physiological function, since it presents two different kinds of scolopidia in one preparation, allowing easy electrophysiological distinction between the few neurons involved.

In the Diptera a pair of tarso-pretarsal non-connective chordotonal organs (one scolopidium each) occur in the blackfly *Simulium vittatum* (Simulidae) (Sutcliffe and McIver, 1987). One scolopidium is in the basitarsus and the other in the fifth tarsomere; each has two neurons. Both scolopidia are attached to the unguitractor tendon proximally and apparently insert onto cellular material on the tarsal wall distally.
In the Orthoptera, two scoloparia in the third tarsomere of the locust *Schistocerca gregaria*, appear to have two neurons each, revealed using CoCl₂ staining (Mücke, 1991) (Fig. 6a). Their ultrastructure was not investigated, but would be of great interest, since tarso-pretarsal chordotonal organs appear to show a consistent trend of comprising small groups of scolopidia of the two basic types (mononematic and amphinematic). Since the relationship of ultrastructure to function is unknown for these types, this represents a likely opportunity for their investigation.

4. Ultrastructure

Gray (1960) was the first to describe the ultrastructure of an insect scolopidium (the mononematic scolopidium of the locust tympanal organ). Howse (1968) and Moulins (1976) reviewed the knowledge of variation in scolopidial ultrastructure. More recent descriptions have added only embellishments to the theme set out by Moulins, rather than major new structures. The one exception is the discovery that, in Collembola, scolopidial structures are associated with exteroreceptive hair sensilla (Altner and Theis, 1984; Crouau et al. 1987), contrary to the case in all other arthropods where scolopidia are always within interoreceptors. The most important advances in ultrastructural research of chordotonal organs have related to the understanding of operational mechanisms.

4.1 General Scolopidial Structure

The following is a review of the ultrastructural organisation of an insect scolopidium, using the terminology and illustrations in Fig. 11a as a guide. The scolopidium comprises an intimate association between one to four bipolar sensory neurons and two special cells that wrap around the dendrite(s): the scolopale cell and the attachment cell. As in other Type I arthropod sensilla, the dendrites form a proximal *dendritic inner segment* (McIver, 1985), and a distal *dendritic outer segment* (McIver, 1985). The latter is a modified stereocilium with a $9 \times 2 + 0$ arrangement of microtubules in the *axoneme* (except in the moth tympanal organ, where the central microtubule pair is present; Ghiradella, 1971). Most of the dendrite is surrounded by the *scolopale cell*. The dendritic inner segment is tightly bound to this cell, but a cylindrical space is created by the scolopale cell around the dendritic outer segment (cilium). The scolopale cell surrounds the cilium and joins upon itself to form a *mesaxon*, leaving an enclosed *scolopale space* which is in communication with the extracellular medium. At the base of the cilium, the dendritic inner segment always contains a pair of centrioles, the *proximal basal body* and the *distal basal body* (which gives rise to the cilium distally). The cilium is prolonged proximally into a *ciliary root* which extends back into the dendritic inner
segment and often well into the neuronal soma. The root usually divides into branched ciliary rootlets. The scolopale cell produces a dense cylindrical sleeve of scolopale rods, which surround the cilium but remain within the scolopale cell appressed to its inner membrane surface. Proximally, the bases of the rods are attached to the dendrite tip by desmosomes. The distal end of the cilium inserts into a dense extracellular cap or tube, as do the scolopale rods. The cilium, then, is thought to be anchored proximally at the tip of the dendritic inner segment, and distally to the cap; thus the cilium is enclosed in a reinforced cylindrical cavity. The cap itself is firmly connected to an attachment cell which extends distally to some moveable part of the inner cuticular surface, either directly or indirectly. Transverse sections through various levels of the scolopidium (Fig. 11d) show how the scolopale cell envelops the dendrites of two neurons to form a mesaxon, and how the cilia are surrounded by the extracellular scolopale space reinforced by the scolopale rods.

Table 4 summarises the terminology used by different authors for the structures of the scolopidium. Two authors described the scolopale space as a receptor lymph cavity (Rezeptorlymphraum = receptor lymph space), implying a possible ionic regulatory function analogous to that discussed for the receptor lymph cavity of hair sensilla (Thurm and Küppers, 1980). This possibility is explored in Sections 5 and 6.

4.2 METHOD OF FIXATION AFFECTS ULTRASTRUCTURE

Most studies of scolopidial ultrastructure have been based upon chemically fixed material (typically using 2-4% glutaraldehyde as the main fixative). However this method of fixation inevitably leads to fixation artifacts in tissue compartments surrounded by large extracellular fluid spaces, due to bulk water movement into or out of cells during the relatively slow penetration of glutaraldehyde (Steinbrecht, 1992). The result is unpredictable distortion of membrane-delineated structures and inclusion of extracellular bits of membrane in the scolopale space. These artifacts are never seen in the same area in cryofixed material (U. Wolfrum, pers. comm.). A successful solution to the problem is to use rapid cryofixation, followed by freeze-substitution and room-temperature embedding; or to use cryoembedding and cryomicrotomy (reviewed by Steinbrecht and Zierold, 1987). With this method, dendrites of insect scolopidia and cuticular sensilla are oval or circular in cross section, membranes are smooth and evenly contoured, and microvilli and microlamellae of accessory cells are straight and regular (Steinbrecht, 1992; Wolfrum, 1990). In terms of scolopidial ultrastructure, wrinkled and irregular outlines resulting from chemical fixation are most likely to bear upon interpretation of the nature of the ciliary dilation and bending of the cilium, the shape of the dendritic inner segment, and the appearance of extracellular material within the scolopale space. Careful examination of these structures with rapid cryofixation in different scolopidial types is currently required, and
the challenge is to find chordotonal organs small enough and close enough to the cuticular surface to be within the zone of cryofixation (about 10 µm).

4.3 THE BIPOLAR SENSORY NEURON

The neurons in chordotonal organs are always of Type I (see Introduction), with a uniterminal dendrite terminating in a modified cilium. As in most other arthropod sensilla, the cell soma is peripheral. The cytoplasm of scolopidial neurons appears typical of that found in other insect sensory neurons, although virtually nothing is known of its molecular composition. The dendritic inner segment contains abundant mitochondria, microtubules and ciliary rootlets. Microtubules are very densely packed and longitudinally oriented within the dendrite (e.g. Bromley et al. 1980). The number of mitochondria decreases as the ciliary rootlets coalesce distally into the main root, leaving only a few very large mitochondria (Ball, 1981). Mitochondrial size and distribution in this region (e.g. evidence of an abundant supply of metabolic energy) could support Wolfrum’s (1991c) suggestion that the ciliary rootlets actively contract.

4.3.1 Dendrite morphology

Typically the dendritic inner segment terminates as a truncated cylinder which abruptly gives rise to the much narrower cilium distally (Fig. 12a). In
Fig. 11. Diagrammatic structure of mononematic and amphinematic scolopidia, with cilia of type 1 (a,d), type 2 (c) and both types (b). a. Mononematic scolopidium with a single bipolar neurone surrounded by a glial cell proximally and a vacuolated scolopale cell distally. The dendrite consists of an inner dendritic segment and an outer dendritic segment or cilium, which contains a dilation and inserts distally into an extracellular cap. From the cilium, a ciliary root arises proximally at the level of the proximal and distal basal bodies. The scolopale cell secretes the scolopale and creates the scolopale space. The scolopale rods form a cage around the cilium between the inner dendritic segment and the cap. The cap is secured to the microtubule-filled attachment cell. The levels of three transverse sections shown in d are indicated by dashed lines di-iii. b. An amphinematic scolopidium with two neurones, one with a type 1 cilium and the other with a type 2 cilium. The latter is characterised by a highly-reduced ciliary root and a dense terminal tubular body. An electron-dense tube surrounds the cilia and extends through an attachment cell to insert into the cuticle amongst hypodermal cells. c. An amphinematic scolopidium with type 2 cilia and different mode of insertion into the cuticle (ultrastructure shown in Fig. 17). The cilia have unusual shapes with thick distal portions. The largest inserts into a hollow cone that is embedded in the cuticle and separate from the tube. d. Transverse sections of a scolopidium, taken at the levels indicated in a, but showing the more common condition of two neurones per scolopidium. Note how the enveloping cells at each level join upon themselves with a mesaxon junction. a, redrawn from data in Bromley et al. (1980); b, redrawn from data in Schmidt (1969); c, modified from Courbière-Tichané (1971), with permission; d, modified from Moulins (1976), with permission.
Table 4. Terminology used for structures of insect scolopidia (modified from Moulins, 1976)

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<tr>
<td>Centriolar derivative cell</td>
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<td>Dendritic outer segment Attachment cell</td>
<td>Cilium</td>
<td>Segment ciliare</td>
</tr>
<tr>
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<td>Attachment cell</td>
<td>Attachment cell</td>
<td>Hölzelle, Kappenzelle</td>
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<td>Scolopale cell</td>
<td>Scolopale cell</td>
<td>Stiftzelle</td>
<td>Cellule scolopale</td>
</tr>
<tr>
<td>Scolopale Tube</td>
<td>Scolopale</td>
<td>Scolopales Dendrite sheath</td>
<td>Wandrippen Stift</td>
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<td></td>
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chordotonal organs associated with hearing and vibration detection (tibial tympanal organ: Ball, 1981; abdominal tympanal organ: Gray, 1960; subgenual organ: Moran and Rowley, 1975; tymbal organ: Young, 1975), the tip of the dendrite forms a swollen bulb just below the base of the scolopale (Fig. 12b). A bulb is not seen in any of the Type 1 or Type 2 scolopidia in Johnston’s organs of insects, even though many are almost certainly antennal vibration detectors (McIver, 1985). There are significant differences in the frequencies and probably also the amplitude of vibration experienced by these different groups of scolopidia (McIver, 1985; Dambach, 1989). It remains to be investigated whether the bulb provides any functional advantage (perhaps through additional surface area at the dendritic tip).

4.3.2 Basal bodies

Two centriole-like structures, the proximal and distal basal bodies, occur below the base of the cilium, in the tip of the dendritic inner segment (Fig. 13a,b). These are composed of nine triplets of microtubules (Fig. 14d-f) arranged in a cylinder (Moulins, 1976). From the proximal basal body the ciliary root extends proximally, and from the distal basal body the axoneme of the cilium extends distally. In transverse section, the proximal basal body appears stellate, as the ciliary root divides into nine fibre bundles and passes around the periphery of the basal body (Ball, 1981). The distal basal body forms a dense cylindrical base of the cilium, sometimes lined internally with a lattice of dense material in longitudinal section (Figs 13a, 15). At this level the axoneme arises first as a circle of microtubules surrounding a hollow core from which dense material radiates outward to form nine alar spokes (Figs 14e,f, 15) (Young, 1973; Michel, 1974; Ball, 1981). The axoneme proceeds distally as nine pairs of microtubule doublets, arranged in a circle as seen in motile cilia (central doublets missing).

There is little variation in basal body structure across the wide variety of scolopidia examined, except for one report of an enormous basal body in scolopidia of Johnston’s organ in a simuliid fly (Sutcliffe and McIver, 1987).

4.3.3 Ciliary root and rootlets

A single cross-banded ciliary root extends proximally from the proximal basal body (Moulins, 1976). Moving proximally from its array of nine fibrous bundles that pass outside the basal body, the ciliary root takes on a fused cylindrical shape and then subdivides into several to many ciliary rootlets. These can extend the length of the dendrite, soma and sometimes even into the axon, which is farther than ciliary roots in other arthropod sensilla (Moulins, 1976); this may support the proposal that the root has a mechanical anchoring function (e.g. Moran and Rowley, 1975; Wolfrum, 1991a).
Fig. 12. Longitudinal sections (slightly oblique) through the scolopale region of chordotonal sensory dendrites. 

a. Moth wing-hinge chordotonal organ dendrite. Arrowheads mark the apposing membranes of the proximal extensions of the attachment cell and the distal extensions of the scolopale cell. The latter contains scolopale rod material. The asterisk marks rodlike material within the attachment cell. A local ciliary bend can be seen between the dendritic apex and the granular material of the lumen. Cp, cap; G, granular material within the extracellular lumen; Rd, scolopale rod material.

b. Low magnification view of scolopidium, from a New Zealand weta tympanal organ, showing overall organisation of the scolopale region. Note vacuolated appearance of the scolopale cell (S) and the relationship of this cell and the attachment cell (AtC) to the scolopale and to each other. Scolopale cap (SC) also appears vacuolated, as if from highly-folded, electron-dense material. The bulbous apex (DD) of the dendrite (D) is characteristic of sound- and vibration-sensitive scolopidia. CR, ciliary root; M, mitochondria; PS, proximal extracellular space; SE, extension of scolopale cell cytoplasm; SP, scolopale cell projection; SR, scolopale rod. Scale: a, 0.82µm; b, 1µm. a, Yack and Roots (1992), with permission; b, Ball (1981), with permission.
4.3.3.1 Structural variation The size and length of the ciliary root varies in different scolopidia. In the amphineumatic scolopidia of Johnston’s organ each scolopidium has three neuronal dendrites: usually one gives off a thick cilium and the other two give off thin cilia (Howse, 1968; Bode, 1986). The root in the large cilium usually is reduced or absent (e.g. *Chrysopa*: Schmidt, 1969; *Speophyes*: Courbière-Tichané, 1975). In thrips (Thysanoptera), four variations occur, including a large cilium with no root; an unusually short, small cilium with no solid root; thin cilia with normal roots; and thin cilia with weakly-developed roots (Bode, 1986). Johnston’s organ in the termite *Zootermopsis angusti* has amphineumatic scolopidia with a well-developed root in the thick cilium and in one thin cilium, and a very slender root in the other thin cilium (Howse, 1968). In *Monobella grassei banyulensis* (Collembolla), an unusual amphineumatic scolopidium associated with the pretarsal cuticular setae has one cilium with a long, thick root (solid, circular cross-section), and two cilia with short roots which are flat in cross-section (Fig. 18). Also in *Monobella*, the tibiotarsal-pretarsal chordotonal organ has amphineumatic scolopidia with only two dendrites each: one has a long, flat, recurved root and the other has a short, flat root (Crouau et al. 1987). Further differences in roots of the mononematic scolopidia in the tibio-tarsal connective chordotonal organ of the cockroach (Young, 1970). One of the two dendrites of each scolopidium contains a thick (up to 1 µm diameter) hollow root which extends deeply into the neuron, while the other dendrite has only thin rootlets which never fuse into a root proximal to the basal bodies, and which are shorter than the former. The foregoing differences suggest variation in the function of the ciliary root and rootlets in scolopidia, but no experimental investigations have been made to combine physiological and ultrastructural techniques in attempting to relate...
Fig. 13. Ultrastructure of dendritic segments. a. Longitudinal section of a sensory dendrite (SD) and sensory cilium (SC), showing the proximal basal body (pb) and distal basal body (db). Note the regularly-spaced particles (arrowheads) lining the inner surface of the dense axonemal microtubules which arise from the distal basal body, and the banded appearance of the ciliary root as it descends from the distal basal body and passes outside the proximal basal body. b. Similar longitudinal section but showing the commonly-seen bend in the base of the cilium and axoneme, which distorts the apical membrane of the inner dendritic segment and the ciliary root. Neurones in a and b are from the antennal connective chordotonal organ of the cockroach. c and d. Transverse sections of a ciliary root in a grasshopper femoral chordotonal organ. c. Evidence for filamentous connections joining microtubules to adjacent microtubules (arrowhead), to ciliary rootlets (R), and to the cell membrane (arrows). Note the tendency for microtubules to be uniformly spaced away from the ciliary rootlets. d. Section through a different dendrite of the same chordotonal organ, showing bridge-like connections between microtubules and a mitochondrion (m), and connections between microtubules and the cell membrane (arrows). The ciliary root (R) has not yet subdivided into separate rootlets at this level. Scale bar: a, b, d, 0.27µm; c, 0.29µm. a and b from Toh and Yokohari (1985), with permission; c and d from Moran and Rowley (1974), with permission.
structure to function. A powerful approach to solving the function of ciliary roots could incorporate *Drosophila* with mutations in scolopidial composition.

4.3.3.2 Chemical composition Most ultrastructural studies have shown periodicity of cross-banding along the ciliary root fibres. Measurements range from 60 to 68 nm in chordotonal scolopidia (compared to 55 to 75 nm in arthropod sensilla and from 21 to 900 nm in motile and non-motile cilia) (Ball, 1981; Wolfrum, 1991a). Several authors have suggested that the material in the root is collagen (Füller and Ernst, 1973; Michel, 1974; Schmidt, 1969, 1974), since the periodicity of vertebrate D-periodic collagen is 67 nm. However, D-periodic collagen microfibrils always form in the extracellular space after an initial secretion of procollagen by cells. This argues against the presence of collagen in ciliary roots, which are intracellular (Wolfrum, 1991a). Moreover, scolopidial ciliary rootlets are not digested by collagenase, and they do not react with antibodies against vertebrate Type I collagen.

Evidence for a possible contractile role of ciliary roots has come from immunohistochemical localisation of two proteins in the rootlets: α-actinin-like protein (Plate 1a,b) and centrin-like protein (Plate 1c,d) (Wolfrum, 1991a, 1992). In skeletal muscle, α-actinin is an anchoring protein for actin filaments and other filamentous proteins that influence elasticity associated with the Z-line. Centrin is a contractile protein which is known to be the major component of ciliary and flagellar rootlets of motile green algae. It is thought to be responsible for contractions of green algal ciliary rootlets observed in the presence of raised Ca²⁺ concentrations, and is therefore implicated in a possible contractile role in scolopidial ciliary rootlets (Wolfrum, 1991a,c). A possible test of Wolfrum’s hypothesis is to observe the cross-banding period of ciliary roots fixed in different concentrations of Ca²⁺.

4.3.3.3 Are rootlets anchored in cytoplasm? It is not known whether rootlets are somehow anchored in the cytoplasm, and serve to stabilise the cilium
mechanically, but this is suggested by the invasion of long ciliary rootlets well down into the cell soma. Two kinds of possible evidence exist for anchorage along the length of the rootlets. First, the dendritic inner segment is replete with longitudinally-oriented microtubules, which could serve as a cytoskeleton, and profiles in transverse sections of the dendritic inner segment show the solid root or rootlets separated from microtubules by a clear halo of variable width (Fig. 13c). This is common in locusts, beetles, wetas, aphids and collemboles (Courbière-Tichané, 1971, 1975; Moran and Rowley, 1974, 1975; Bromley et al. 1980; Ball, 1981; Altner and Theis, 1984). In *Speophyes lucidulus*, dendritic cytoplasm is well-packed with microtubules but shows clear zones (about 80 nm wide) devoid of any detectable material, delineated with a line of closely spaced microtubules around each ciliary root (Courbière-Tichané, 1971). In aphids and wetas, the microtubules are very densely packed in the dendrite and are much closer to the ciliary root, yet a distinct clear outline surrounds each root (Bromley et al. 1980; Ball, 1981). In the above examples, the presence of the halo suggests that some material may have occupied the empty space (thus excluding microtubules from the vicinity of the root) but was subsequently lost in the preparation of the specimens. Such material could serve to cross-link rootlets to microtubules.

The first evidence for such rootlet anchorage material was shown in the grasshopper femoral chordotonal organ (Moran and Rowley, 1974). Fine filamentous strands radiate out from ciliary rootlets to microtubules, and also interconnect microtubules to each other (Fig. 13 c,d). The exceptionally clear electron micrographs suggest that the filamentous material forms a network that interconnects microtubules with the ciliary roots, other microtubules, the plasma membrane and soluble elements in the cytoplasm, to create a three-dimensional latticework of cytoskeletal support. Hints of such filamentous material may be seen in longitudinal sections from other species (e.g. *Hemideina crassidens*; Ball, 1981). An elaboration of the scheme occurs in the collembolean antennal scolopidial organ IP-AIII, where the ciliary root is surrounded by a conspicuous tube of two membranes, separated by 5-10 nm, bound on the outside by numerous microtubules (Altner and Theis, 1984). Fine lateral filaments join the root to the inner membrane in both transverse and longitudinal sections. These could serve to anchor the ciliary root to a scaffolding of microtubules.

A second ultrastructural scheme for a possible anchoring mechanism occurs in the locust femoral chordotonal organ (Slifer and Sekhon, 1975) and the moth wing-hinge chordotonal organ (Yack and Roots, 1992). Here the cytoplasmic microtubules crowd directly against the ciliary root and rootlets, and fuzzy or grainy material is seen along the edge of the cross-banded root structure in longitudinal section. This material intermingles between microtubules and the cross-banded edge. In longitudinal section (Fig. 6 in Slifer and Sekhon, 1975), the rootlets appear to transform into microtubules proximally as the cross-banded material becomes progressively thinner. The
thinning is not due to an off-axis section plane because the long microtubules remain in the section.

4.3.3.4 The ciliary collar  Around the distal end of the dendritic inner segment the root appears to be mechanically anchored to the dendritic membrane. Here the root is associated with a set of belt desmosomes (zonula adherens; Moulins, 1976) which bind the bases of the scolopale rods to the dendritic membrane, and appear to send radial filaments toward the root. This attachment zone (the ciliary “collar”) includes the region where the root subdivides into nine fibres around the proximal basal body (Ghiradella, 1971; Yack and Roots, 1992).

The collar takes two forms, which do not seem to be correlated with chordotonal organ type, but which may be separated by insect order. The first form, the laminated collar, is predominant in the Lepidoptera and Neuroptera. It is found in scolopidia of Johnston’s organ *(Manduca sexta; Vande Berg, 1971)*, the thoracic tympanal organ *(Feltia subgothica; Ghiradella, 1971)*, the tibio-pretarsal chordotonal organ *(Tineola biselliella; Fauchuex, 1985)*, the wing-hinge chordotonal organ *(Actias luna; Yack and Roots, 1992)* and the neuropteran subgenual organ *(Chrysoperla carnea; Devetak and Pabst, 1994)*. In the laminated collar, multiple concentric layers line the inner circumference of the dendrite. In *Actias*, ten layers, arranged as four pairs and two separate layers (Fig. 14b,c) appear to connect to the ciliary root by faint scattered filaments (Yack and Roots, 1992). In other species the number of layers ranges between three and five, and can be discontinuous rather than continuously concentric (e.g. Vande Berg, 1971; Fauchuex, 1985). Usually the radial filaments connecting to the root are very faint, although they appear to become heavier between the layers of the collar (e.g. Fauchuex, 1985).

The second form is a non-laminated collar, found in Orthoptera, Blattaria, Coleoptera, Homoptera and Diptera. It occurs in scolopidia of the femoral chordotonal organ *(Chorthophaga viridifasciata; Moran et al. 1975; Romalea microptera: Slifer and Sekhon, 1975; Drosophila melanogaster: Shanbhag et al. 1992)*, the subgenual organ (SGO) *(Periplaneta americana: Howse, 1968; Moran and Rowley, 1975)*, the tymbal organ *(Cyclochila australasiae: Young, 1975)* and the antennal connective chordotonal organ *(Speophyes lucidulus: Courbière-Tichané, 1971, 1975; Periplaneta americana: Toh, 1981)*. Instead of concentric layers, only heavy deposits of electron-dense material are seen on the inner dendritic membrane side of the desmosome. Often prominent radial fibres radiate from the root to the desmosomes (Fig. 14a); these appear paired in the SGO and tymbal organs (e.g. Young, 1975). In other preparations the radial filaments are faint and incomplete, particularly in a halo-like region around the root (e.g. Courbière-Tichané, 1971). A weak concentric network of filamentous material in the dendrite at this level was shown by Shanbhag *et al.* (1992) in the *Drosophila* femoral chordotonal organ.

In the known examples of ciliary collars there is only evidence of a weak
Fig. 14. Ultrastructure of cilium in mononematic scolopidia, progressing distally from the level of the ciliary root (taken from several different examples).  

a. Transverse section through dendrite tip (SD) showing scolopale rods (sr) attaching to dendrite membrane by desmosomes (arrow). Note filamentous bundles (arrowhead) joining ciliary root (cr) and desmosomes.  
b. Laminate belt desmosome forming an octagonal dendritic collar (CI) around the dendrite at the level of the ciliary root. Scolopale rods, enclosed within the scolopale cell membrane, are joined to the dendrite by the desmosome and appear to have a hollow core (star). Asterisk, scolopale space; M, mesaxon of scolopale cell; arrowheads, electron-dense amorphous material.  
c. Detailed view of the dendritic collar showing ten layers (small white arrowheads) arranged into four doublets and two singlets. Dendrite and scolopale cell membranes are indicated by the large black and white arrowheads, respectively.  
d. Proximal basal body. The inner ring of triplet tubules is attached to the nine surrounding ciliary root processes.  
e. Distal basal body at the point where the ciliary shaft emerges from the dendrite surface. Alar spokes (as) are linked by cross-connecting strands (c).  
f. Ciliary base. Axoneme doublets are embedded
connection with large surface area between the ciliary root (or proximal basal body) and the dendritic membrane; the strongest connection appears to be between the dendritic membrane and the scolopale rods. Therefore, models that are based upon a strong anchor-point for the cilium at this level in the dendrite must treated tentatively.

4.3.3.5 The cilium Cilia of Type 1 are of uniform diameter, except for a subterminal ciliary dilation, and the axoneme maintains its 9 + 0 microtubular format, except in the dilation and at the distal tip where the cilium inserts into the cap (Fig. 15). In Johnston’s organ, the Type 1 cilium inserts into a tubular sheath instead of a cap (Figs 11b, 16b). Cilia of Type 2 have two regions: a proximal cilium with an axoneme as above but no ciliary dilation, and a distal, non-ciliary section of enlarged diameter and devoid of the axoneme (Fig. 11c). Instead, the distal section is filled with microtubules, and inserts into a dense tubular sheath (Moulins, 1976). In Type 2 cilia of Johnston’s organ, the apical end of the distal section forms a tubular body of densely packed microtubules (similar to the tubular body seen in hair and campaniform sensilla; Thurm et al. 1983).

The functional difference between Type 1 and Type 2 cilia remains to be

Fig. 14 caption continued.

in a ring of electron-dense material (dr) and connected to the ciliary membrane by radial extensions (re).

g. Ciliary shaft at mid-length. In the axoneme, A-tubules appear solid, each with a pair of arms. B-tubules are hollow and merged to the A-tubules.
h. Ciliary dilation showing close association of axoneme doublets with the ciliary membrane. A central core of granular material has formed. A-tubules have become hollow and lack arms; B-tubules are connected to A-tubules on one side only.
i. Transverse section through the cap (CP) at mid-level showing two cilia tightly apposed to the surface of the narrow cavity. Note the lacunar nature of the cap. Scales (in µm): a, 0.55; b, 0.57; c, 0.2; d, e, f, g; 0.1; h, 0.17; i, 0.5. a, g, h, i, cockroach antennal connective chordotonal organ, from Toh and Yokohari (1985); b, c, moth wing hinge chordotonal organ, from Yack and Roots (1992), with permission; d, e, f, cicada tympanal organ, from Young (1973) with permission.
Fig. 15. Diagrammatic interpretation of ciliary ultrastructure (cicada tympanal organ). Compare with electron micrographs of Fig. 14. Longitudinal view (left) is at half the magnification of the transverse views (right) and distal ciliary shaft and dilation are foreshortened. Modified from Young (1973), with permission.
shown. Moulins (1976) reviewed suggestions that the effective stimulus for Type 1 is either stretching of the ciliary dilation or lateral compression of the apical region, whereas that for Type 2 is thought to involve a difference in relative movement of the tube and the distal ciliary section.

4.3.3.6 Cilium ultrastructure The modified stereocilium projecting from the distal tip of the dendrite differs in a variety of ways from cilia found in other tissues. Research has focused mainly on relating cilium ultrastructure to a possible role in transduction of mechanical stimuli. The ciliary membrane is contiguous with, and apparently not different from, that of the dendritic inner segment, although nothing is known about possible differences in ion channel composition or mechanical sensitivity between these parts of the neuron.

4.3.3.6.1 Type 1 cilia Detailed descriptions of mononematic Type 1 cilia (Figs 15, 16a) have shown that the ultrastructure is more complicated than previously thought. Near the distal basal body, the axoneme is thickened and invested with a heavy electron-dense matrix for the first 700nm of its length. Nine B-tubules are embedded in the matrix, and the internal surface of this cylindrical structure is lined with fine knobs which may represent internal annuli or a spiral (Fig. 13a). Often this thick basal portion of the cilium appears bent in longitudinal sections (Figs 12a, 13b) (Michel, 1974; Toh and Yokohari, 1985; Yack and Roots, 1992). A specialisation at the ciliary base (in a region termed the ciliary necklace in motile cilia) is the presence of radial extensions ("champagne-glass structures") linking the axoneme microtubules to the cillum membrane (Fig. 15). Similar extensions are associated with motile cilia and vertebrate sensory cilia (Young, 1973). In addition, vesicles have been observed along the interior of the cilium (Toh and Yokohari, 1985).

Distal to the base, the axoneme consists of nine microtubule doublets (Figs 14g, 15), each comprising an A-tubule (with an electron-dense core) and a B-tubule (which is hollow). Usually no central microtubules are observed, except in Lepidoptera, where two microtubules occur (tympanal organ: Ghiradella, 1971; pretarsal chordotonal organ: Fauchex, 1985). The A-tubule bears a pair of arms which are presumed to be ATPase dynein (Thurm et al. 1983). Granular material lines the internal surface of the ciliary membrane, linking the membrane to the microtubule doublets at regular indentations of the membrane (Toh and Yokohari, 1985). In transverse profiles, the granular material forms an electron-dense lining of the membrane, from which projects bridging material to the A-tubule arms (Fig. 20b). Crouau (1983) argued that the membrane lining in insect chordotonal cilia obscures a lining of closely-spaced projections apposed to the membrane. In the mysid shrimp Antromysis jubertiei he showed that such projections are clearly seen to give rise to bridging structures linking the ciliary membrane to the A-tubule arms of the axoneme (Fig. 20b). These structures bear a resemblance to the membrane-integrated cones (MIC) described by Thurm et al. (1983) as linking bridges between the membrane and internal microtubules of campaniform sensilla in
Fig. 16. Diagrammatic summary of scolopidial ultrastructure, particularly junctions between and within component cells. Drawings are synthesised from many studies of mononematic and amphinematic scolopidia, to show variety of junctional types described in text. 

**a. Mononematic scolopidium.** Amphinematic scolopidium illustrating: belt desmosome; belt desmosome type II; electron-dense filaments anchoring attachment cell to cuticle; filaments joining ciliary root to belt desmosome at scolopale base; gap junction between adjacent scolopale cells; gap junction type IV; granular material at two characteristic locations within scolopale space; hemidesmosome; laminate desmosomal junction; septate desmosome; septate junction; spot desmosome; MDc, microtubule-associated desmosome within folds of attachment cell membrane. Authorities for junctions numbered above: 

flies. The identity of structures associated with scolopidial ciliary microtubules could be aided by utilising immunological probes against microtubule-associated proteins (MAPs). This could help to resolve various current hypotheses proposing mechanical interactions between structures within the cilia (see Section 5.2).

The *ciliary dilation* occurs some two-thirds of the way along the length of the cilium, and is always characterised by the presence of electron-dense material in its centre (Fig. 14) (Moulins, 1976). The appearance of this material varies widely in transverse section. In some cilia it forms a large, central, circular core (Courbière-Tichané, 1971); in others two to five smaller circular bodies occur in the centre of the dilation and broad, bilayered spokes project inward from each microtubule doublet (Slifer and Sekhon, 1975; Moran and Rowley, 1975); in other cilia a stellate structure extends out to the paired microtubules of the axoneme (Toh and Yokihari, 1985). Another arrangement consists of a central core and a broad ring just inside the axoneme; fine filaments connect these two arrays of dense material (Toh and Yokihari, 1985). Shanbhag *et al.* (1992) described a large, circular density filling the dilation, but the density, in turn, was filled with many small electron-lucent “inclusions”. The dilation can also be filled with amorphous or fibrillar material (Young, 1973; 1975; Slifer and Sekhon, 1975).

Another characteristic of the dilation is a clear connection between each microtubule doublet and the ciliary membrane (Fig. 14h). In some cases, when the axoneme is not as expanded as the cilium, the membrane is pulled in to make contact with the axoneme, resulting in a lobed appearance in transverse section (e.g. Courbière-Tichané, 1971; Moran and Rowley, 1975), thus supporting the conclusion of many authors that the ciliary membrane is firmly attached to the axoneme.

A further characteristic of the dilation is a change in the microtubule appearance (Fig. 14d). First, the dense core of the A-tubule becomes clear. Second, the arms of the A-tubule are lost. Third, the B-tubule often has a horseshoe-shaped cross-section instead of a circular one. Only one of the ends is attached to the A-tubule (Toh and Yokohari, 1985).

All of the above ciliary modifications in the dilation suggest that it is an important part of the specialised scolopidial mechanism for transduction of mechanical energy into a neuronal response; such modifications are only found in cilia of Type 1 scolopidia. Elucidating the function of these elaborate structures poses a unique challenge to modern researchers. Moulins (1976) suggested that the dilation material is comparable to the tubular body of campaniform and hair mechanosensilla of insects, which is sensitive to lateral compression (Thurm *et al.* 1983). Howse (1968) suggested that the material in the dilation acts as a cytoskeleton which resists lateral compression, and thereby causes stretching of the ciliary membrane during axial pull on the scolopidium. However the anchoring of the membrane to the axoneme and to the internal dilation material may well argue against this.
The tip of the cilium distal to the dilation narrows to the original cilium diameter and inserts into the extracellular cap (Fig. 14i). There is conflicting evidence for a special attachment of the cilium to the cap. It is either tightly enclosed by the cap material (Courbière-Tichané, 1971; 1975; Moulins, 1976; Yack and Roots, 1992), or inserted into folds of the dense cap material (Moran and Rowley, 1975; Young, 1975; Ball, 1981; Toh and Yokohari, 1985). The normal axoneme 9 + 0 configuration appears, but the B-tubules may have the broken appearance seen in the dilation (Toh and Yokohari, 1985). The tips of some cilia terminate in a swollen bulb within or distal to the cap (Courbière-Tichané, 1971, 1975; Schmidt, 1969), which may be filled with tiny vesicles (Yack and Roots, 1992) or may contain an electron-dense spheroid several times the diameter of the cilium (Toh and Yokohari, 1985). In the subgenual organ of Periplaneta americana, the cilium forms an elongate swelling within the cap (Moran and Rowley, 1975). The bulb or swelling within the cap may serve as a means of mechanically anchoring the cilium in the cap.

Amphinematic Type 1 cilia are found in tibio-tarsal and pretarsal chordotonal organs and Johnston’s organs (Figs 11b, 16b). They differ from the mononematic type by inserting distally into a tube or sleeve of electron-dense material; usually the thickest one extends further than the others (Moulins, 1976; McIver, 1985). No special junction occurs between the cilia and the tube. Sometimes the cilia fit snugly into the tube (Vande Berg, 1971) but often there is a considerable gap between the structures (Courbière-Tichané, 1971, 1975), leading Moulins (1976) to claim that the cilia are not firmly attached to the tube in amphinematic scolopidia. The ultrastructure also differs slightly from that of the mononematic type: (a) the ciliary root and basal bodies may be smaller in diameter and shorter than those in mononematic scolopidia (Bode, 1986), (b) the axoneme contains the typical 9 x 2 + 0 configuration and expands in the ciliary dilation, (c) the ciliary dilation contains a large electron-dense body filling the dilation space and enclosing the microtubules (Schmidt, 1970; Bromley et al. 1980; Boo and Davies, 1980), (d) the axoneme distal to the ciliary dilation becomes disorganised into an array of single microtubules (Moulins, 1976), which in aphids may develop into an electron-dense tubular body at the tip (Fig. 16b) and dipterans (e.g. Boo and Davies, 1980).

4.3.3.6.2 Type 2 cilia Amphinematic Type 2 scolopidia occur primarily in Johnston’s organ (of which McIver lists a total of 37 studies as of 1985). Type 2 ciliary structures occur also in mouthpart chordotonal organs (Moulins, 1976), tibio-tarsal and pretarsal chordotonal organs (Bode, 1986), and, for the first time, in association with cuticular hair sensilla which are innervated by chemoreceptor neurons (Fig. 18). The basic ultrastructural plan is shown in Figs 11c and 17. Proximally, the ciliary segment arises from a distal basal body in the dendrite, but ciliary roots are either diminutive or absent (Fig. 17a) (Schmidt, 1970; Courbière-Tichané, 1971; Bode, 1986). The proximal region of the ciliary segment has the usual axoneme structure (Fig. 17b), but Type 2 cilia undergo a transition from the proximal axoneme (9 x 2 + 0) to a distal...
prolongation more or less filled with single microtubules (Fig. 17c,d) which, in the case of Johnston’s organs, may form a dense tubular body at the extreme tip (Schmidt, 1969; Courbière-Tichané, 1975). The distal microtubular segment is not always enlarged in diameter, but it invariably is ensheathed by an electron-dense tube closed at the distal end and attached directly to the cuticle (Fig. 17d-f). Type 2 cilia never have a ciliary dilation filled with electron-dense material, as seen in Type 1 cilia (Moulins, 1976).

The scolopidia of Johnston’s organs represent the greatest diversity of ciliary structures found in insect chordotonal organs. Type 2 cilia in these organs occur together with one or two Type 1 cilia inserting into a common cuticular tube. Bode (1986) reported five different kinds of arrangements of cilia in Johnston’s organ of Thysanura (Thrips validus), whereas Diptera have three (Simulium) and sometimes four (Anopheles) arrangements (A, B, C and D; McIver, 1985). The cilium has a greater diameter than the Type 1 cilium in Johnston’s organ (the “thick” vs “thin” cilium of Schmidt, 1969) and, although Schmidt (1970) and Bode (1986) show a dilation midway along the cilium, it is never filled with electron-dense material. There is no ciliary root in the dendritic inner segment. In Johnston’s organs of thrips the apical tips of the Type 2 ciliary segments have no electron-dense material between the microtubules and hence lack a typical tubular body (Bode, 1986), whereas those of Chrysopa and the beetle Speophyes have a tubular body (Schmidt, 1969; Courbière-Tichané, 1975).

Type 2 cilia do not appear to be firmly attached apically within the sheath or tube (Moulins, 1976; Courbière-Tichané, 1971). This is likely to affect the way in which these sensilla transduce mechanical stimuli, and is clearly different from cases in Type 1 cilia where the tip is tightly apposed to the cap (Moulins, 1976).

4.3.3.7 Mechanical attachments between cilia within scolopidia In most insects the three cilia of Johnston’s organ are not connected, but in the beetle Speophyes and in Thrips validus (Bode, 1986) a gap junction joins the single Type 2 cilium with both Type 1 cilia (the Type VI junction of Courbière-Tichané, 1975) (Fig. 16b). Four to five layers of electron-dense material occupy the extracellular contact zone along most of the length of the three cilia, and are reminiscent of the laminated junction seen in ciliary collars of the moth wing-hinge chordotonal organ (Courbière-Tichané, 1971; Moulins, 1976; Yack and Roots, 1992). The junction material appears to form a matrix with fine transverse fibres between the cilia. Dense material lies on the inner side of the cilia membranes and encloses the microtubules of the Type 2 cilium. Inasmuch as the two Type 1 cilia are not attached to a cap, the laminated junction may provide a distal mechanical anchorage for these cilia (Moulins, 1976).

4.3.3.8 Bending and distortion of cilia shape Often a distinct bend is observed at the proximal end of a cilium, in the region of the distal basal body (Figs 12a, 13b). This has been seen in Johnston’s organs of Chrysopa (Schmidt,
Fig. 17. Ultrastructure of amphinematic scolopidium with type 2 cilia (beetle mandibular chordotonal organ, illustrated schematically in Fig. 11c). a. At the level of contact between the scolopale (s) and two proximal dendritic segments (d), only scattered, curved electron-dense masses (edm) are seen, rather than ciliary roots. Desmosomes (ds) join the dendritic membranes to the scolopale cell. b. Distal to the dendrite, the two cilia (c) initially contain axonemes, and are surrounded by the scolopale and vacuolated scolopale cell. c. Still within the scolopale space, the axonemes in the cilia are replaced by numerous microtubules, and one cilium becomes enlarged (lc). At the point of contact the cilia are fused together. d. More distally, the small cilium terminates and the larger one (lc) becomes enclosed in an electron-dense tube (t). e. Immediately upon penetrating the cuticle, the tube is replaced by a larger, thicker-walled cone, and the large cilium narrows. f. At the apex of the cone the internal cavity closes and the cone is tightly embedded in the cuticle of the mandible. Scales: a, c, e, 0.3µm; b, 0.5µm; d, 0.2µm; f, 1µm. a-f, Courbière-Tichané (1971), with permission.
1969) and *Simulium vittatum* (Boo and Davies, 1980), the tympanal organs of *Gryllus bimaculatus* (Michel, 1974) and *Hemideina crassidens* (Ball, 1981), the antennal connective chordotonal organ in *Periplaneta americana* (Toh and Yokohari, 1985), the femoral chordotonal organs of *Carausius morosus* (Füller and Ernst, 1973), *Chortophaga viridifasciata* (Moran and Rowley, 1975) and *Romalea microptera* (Slifer and Sekhon, 1975), and the wing-hinge chordotonal organ of *Actias luna* (Yack and Roots, 1992). Presumably the cilia bend upon fixation. It should be noted, however, that in heterodynal scolopidia both cilia are not always bent (e.g. Slifer and Sekhon, 1975). This could be a functional correlate of the observation that individual cilia within a scolopidium often have differences in the dilation, basal body and rootlet ultrastructure (Young, 1970; Ball, 1981; Toh and Yokohari, 1985). Alternatively, cilia of mononematic Type 1 scolopidia could undergo active ciliary bending during transduction of mechanical stimulation, as hypothesized by Moran *et al.* (1977) and discussed further in Section 5.2.

In another form of distortion (tympanal organ of the weta *Hemideina* and in the femoral chordotonal organ of *Chortophaga*) the cilia are bent in at least two places as they pass around cytoplasmic extensions of the scolopale cell within the scolopale space (Moran *et al.* 1977; Ball, 1981). Although it is not clear whether the cilia normally function in this state, the observation has implications for the controversial question (Section 5.2) of whether cilia can maintain stiffness to mechanically transmit force from the cap to the dendritic inner segment or whether they actively slide even in a bent position (Moran *et al.* 1977).

### 4.4 THE SCOLOPALE CELL

The scolopale cell wraps around the distal tip of the dendritic inner segment (forming a mesaxon or septate junction), encloses the cilium in the scolopale space, attaches to the distal cap or tube, and produces the intracellular scolopale (Fig. 11d). The region of the cell surrounding the cilium is differentiated into a highly vacuolated cytoplasm, whereas the more proximal cytoplasm of the cell has a dense appearance, and is rich in microtubules (Ball, 1981; Devetak and Pabst, 1994).
4.4.1 The labyrinth

The extensive vacuole system surrounding the scolopale space and cilium (Figs 12b, 16b) is actually a labyrinth of extracellular cavities which communicate with the scolopale space, implying that the scolopale cell could be secretory in nature (Young, 1970; Moulins, 1976). The scolopale cell is thought to be homologous to the trichogen cell of campaniform and hair sensilla (for conflicting evidence see Schmidt, 1973), in which there is evidence in aldehyde-fixed specimens for secretion of acid mucopolysaccharide-like material into the space surrounding the cilium. If such material were secreted by the scolopale cell, it could play a role in ion regulation, as hypothesised by Thurm and Küppers (1980) for hair sensilla, and Treherne et al. (1982) for axons in the central nervous system. A glial glycoprotein (glionexin) was recently demonstrated to surround scolopale cells of the subgenual, tympanal and intermediate organs in the cricket *Acheta domesticus*, and one of its suggested functions was ion regulation in the transduction region of the scolopidium (Field et al. 1994). Glionexin deposited in the extracellular matrix (ECM) could act as an ionic reservoir for sodium in the same way as ECM mucopolysaccharides are thought to act as an anionic matrix which sequesters sodium ions to be released in the face of changes in ionic composition of the extracellular fluid. The function of the labyrinth and the possible role of the scolopale cell in ionic regulation requires further investigation.

4.4.2 Intercellular junctions

Junctions in mononematic and amphinematic scolopidia from a variety of sources are summarised in Fig. 16a,b.

At its proximal end, the scolopale cell tightly encloses the dendritic inner segment and is attached to it by elaborate belt desmosomes (zonula adherans) on the inner contact face of each scolopale rod. This junction is very similar in mononematic and amphinematic scolopidia (Courbière-Tichané, 1971, 1975; Boo and Davies, 1980). The desmosomes are thought to provide a firm mechanical anchorage of the base of the scolopale to the dendritic membrane (Moulins, 1976; Moran et al. 1977), and also could anchor the ciliary root to the dendritic membrane via the ciliary collar (Section 4.3.3.4). The scolopale cell forms spot desmosomes, hemi-desmosomes and gap junctions with neighbouring scolopale cells, and with glial cells, in mononematic but not in amphinematic scolopidia (Young, 1970; Moulins, 1976; Ball, 1981; Toh, 1981; Devetak and Pabst, 1994). At its distal end the scolopale cell makes lateral contact with the attachment cell by multiple septate junctions (septate desmosomes of Young, 1970; Toh and Yokohari, 1985) and distal contact with the cap or tube. If the contact is with a cap (mononematic scolopidium), the scolopale cell forms hemi-desmosomes (or tight junctions; Yack and Roots, 1992), but if it contacts a tube (amphinematic scolopidium) there is no
Fig. 18. The pretarsal seta of a collembolan are unusual cuticular sensilla in that they are innervated by a neurons associated with a scolopale. a. One neurone (1) has a large ciliary root in the dendritic inner segment. Two other neurones (2,3) innervate the seta, both with short ciliary roots. b. Transverse section at the level indicated by the dashed line. The three cilia are ensheathed in a tube and joined together by desmosomes before entering the seta. c. Two of the cilia, from neurons 1 and 2 have axonemes like those seen in chordotonal sensilla. The third, contains scattered microtubules. Note that the scolopale cell surrounds all three neurons but the scolopale does not. Modified from Crouau et al. (1987).

specialised anchoring mechanism (Moulins, 1976). The implications of this difference are discussed in Section 5.

4.4.3 The scolopale

The scolopale was thought to be unique to chordotonal sensilla (Moulins, 1976) but it is now known to occur in collembolan hair sensilla (Fig. 18) and
insect thermo- and hygrosensitive sensilla (Altner and Theis, 1984; Altner and Loftus, 1985; Crouau et al. 1987) which appear to be intermediary forms between hair and chordotonal sensilla types. The most outstanding feature of the scolopale is that it is an intracellular structure which is not homologous to the extracellular sheath or tube of other sensilla. It is a cylindrical structure produced by the scolopale cell immediately against the plasma membrane delineating the scolopale space around the cilium. Proximally it is secured to the tip of the inner dendritic segment, and distally it inserts into the cap (Fig. 17).

The shape of the scolopale can range from a continuous cylinder with few or no fenestrations (e.g. cicada tymbal organ; Young, 1975) to the more commonly seen cylindrical array of rods extending from the inner dendritic segment towards the cap, where they fuse before contacting the cap. In the latter case, the scolopale cell labyrinth opens directly into the scolopale space between the rods (Moulins, 1976; McIver, 1985). In most scolopidia, the rods are of even thickness and tapered at both ends (see Howse, 1968 for a variety of illustrations), but those in cricket tympanal organs are of uneven thickness along their length and may be grouped consistently into subtypes by this criterion (Fig. 19a-d) (Young and Ball, 1974a). In fact, the scolopales of many sound and vibration-sensitive chordotonal organs have a thick belt midway
along the scolopale with a thin band on either side. These particular scolopidia also contain inner dendritic segments with a bulbous apex (Fig. 19). A functional interpretation of this suite of scolopidial traits could be investigated with a combination of ultrastructural, immunocytochemical and electrophysiological approaches. For example, a tonotopic analysis of the subtypes described by Young and Ball (1974a) would allow the correlation of ultrastructure with specificity of response.

Some scolopales are disorganised or incomplete. In the moth wing-hinge chordotonal organ the scolopale material consists of scattered bundles which tend to form a cylinder in the cap region but become more disorganised proximally (Fig. 12a). Some of the material is coalesced around the scolopale space while other bundles are distributed through the scolopale cell cytoplasm. At the level of the proximal dendritic segment, many of the bundles fuse into eight rods attached to the elaborate laminated collar by a belt desmosome (Fig. 14b) (Yack and Roots, 1992). In collembolan scolopidia associated with external cuticular sensilla, the scolopales occur as two or three rods on one side of the scolopale space only (Altner and Theis, 1984; Crouau et al. 1987). This reduction must mean that the scolopale cannot efficiently serve its commonly-suggested role of mechanically reinforcing the scolopale space against deformation.

The number of scolopale rods varies amongst different chordotonal organs, and seems to bear little correlation to chordotonal organ type, location, or insect species. The following have been observed: tympanal organ (and crista acustica), 5-8 (Ball, 1981); subgenual organ, 5-7 (Howse, 1968; Moran and Rowley, 1975; Devetak and Pabst, 1994); antennal connective chordotonal organ, 3-10 (Toh, 1981; Wolfrum, 1990); femoral chordotonal organ, 5-9 (Füller and Ernst, 1973; Slifer and Sekhon, 1975; Moran et al. 1975; Shanbhag et al. 1992); wing-hinge chordotonal organ, 5-8 (Möss, 1971; Yack and Roots, 1992); tarsal chordotonal organ, 8 (Wiese and Schmidt, 1974); Johnston’s organ, 7-8 (Vande Berg, 1971).

4.4.3.1 Chemical composition Scolopale material appears as densely-packed microtubules surrounded by filaments composed of filamentous actin 7-10 nm in diameter, as demonstrated by phalloidin immunolabelling (Plate 1) and high pressure freezing electron microscopy (Wolfrum, 1990). The actin is thought to act as a cytoskeletal supporting framework for the microtubules, since the actin polarity is unidirectional and is therefore unlikely to be used for contraction with any myosin-like system. Tropomyosin has also been co-localised with actin in scolopales, and may act as a stabilising structure for the actin filaments (Wolfrum, 1991b). Since tropomyosin reversibly binds to actin through a calcium-regulated calmodulin/caldesmon system, Wolfrum (1991b) has suggested that the scolopale is an elastic structure that can restore its mechanical distortion with a Ca\(^{2+}\) regulated variability, and hence regulate receptor sensitivity.
The microtubules in scolopales of *Periplaneta* and *Schedorhinotermes* do not show immunoreactivity to an anti-α-tubulin monoclonal antibody (Wolfrum, 1990).

4.4.4 The scolopale space (receptor lymph cavity)

The space or lumen enclosed by the scolopale cell around the cilium is not devoid of contents. Moulins’ (1976) suggestion that the scolopale cell could be secretory is substantiated by observations of lightly-staining, granular or amorphous material distributed throughout the scolopale space (Young, 1975; Wolfrum, personal communication). However it also may form a discrete band midway along the space in longitudinal sections of scolopidia (Figs 12a, 16a). (Moran and Rowley, 1975; Moran *et al.* 1975; Michel, 1977; Toh and Yokohari, 1985; Ball, 1981; Bloom *et al.* 1981; Yack and Roots, 1992; Devetak and Pabst, 1994). The band is either granular and amorphous (e.g. Yack and Roots, 1992) or a web of fibrils (Toh and Yokohari, 1985; Shanbhag *et al.* 1992). Ball (1981) described it as an extension of the scolopale cell into the scolopale space, but this has not subsequently been confirmed. The cilium appears to be restricted from lateral movement by the granular band, if interpretations of bending proximal and distal to the band (Yack and Roots, 1992) are applicable to the living scolopidium.

A second region of granular material overlies the distal tip of the dendritic membrane which is thought to be depolarised by bending of the cilium in mononematic scolopidia (Figs 12, 16a) (Moran *et al.* 1977; Toh and Yokohari, 1985; Yack and Roots, 1992). The suggestions, mentioned above, that accumulations of acid mucopolysaccharides and other glycoproteins could act as ionic regulators may also apply to this granular region in the scolopale space.

Membranous material and vesicles may occur in the scolopale space. These are irregular in outline and often appressed together as if collapsed (Füller and Ernst, 1973; Slifer and Sekhon, 1975; Toh and Yokohari, 1985). Although they could be longitudinally extending fingers of the scolopale cell, they appear devoid of material within.

4.4.5 The scolopale cap or tube

The cap characterises mononematic scolopidia and the tube characterises amphinematic scolopidia (Fig. 16a,b). The cap occurs in two forms. First and most often, it is composed of a highly-folded electron-dense material, giving it a spongy appearance (see especially Fig. 2 in Ball, 1981). One extreme case of reduction in amount of folding is observed in cicada tymbal organs, where four folds radiate out from a hollow centre which contains the cilium (Young, 1975). Second, in some preparations the cap appears to be a solid, amorphous structure lacking internal folds. In this case, if the cap is extremely
electron-dense (Courbière-Tichané, 1971; Moulins, 1976; Devetak and Pabst, 1994), it could be composed of such highly-compacted folds that it appears solid. However, in other cases, it is a lightly-staining mass which is clearly devoid of folds and thus has a different construction from that seen in spongy caps. In the moth wing-hinge chordotonal organ (Yack and Roots, 1992) the amorphous material is filled with abundant circular “pores” (which could be equally interpreted as microtubules in transverse section).

The structure and mode of ensheathing the cilium differs in the cap and the tube. In a cap, the cilium is firmly attached to, or at least closely contacted by, the cap material, and may penetrate the cap to form a terminal bulb (Courbière-Tichané, 1971; Young, 1975; Devetak and Pabst, 1994). If there are two cilia per scolopidium, each is separately enclosed by the cap material. In a tube, there are usually several cilia within the tube lumen and none is closely attached to the tube wall (Moulins, 1976). This difference bears directly on models of mechanical transduction, as discussed in Section 5.

The cap and tube are well endowed with hemi-desmosomes connecting to surrounding cells (Fig. 16). The scolopale cell may extend protrusions, containing the scolopale rods, into the cap (Moulins, 1976). The hemi-desmosomes of the cap give rise to microtubule bundles in the attachment cell.

The origin of the cap and tube is disputed; they may be derived from either the scolopale cell (Moulins, 1976; Blöchl and Selzer, 1988; Yack and Roots, 1992), or from the attachment cell (cap cell) (Ghiradella, 1971; Füller and Ernst, 1973; Moran and Rowley, 1975). Vesicles in the scolopale cell containing material with an electron density similar to that of the cap material may be evidence that the scolopale cell secretes the cap (Yack and Roots, 1992). The attachment cell

4.5 THE ATTACHMENT CELL

The attachment cell or cap cell serves as an apical anchor for the scolopidium. It surrounds the cap as well as the distal portion of the scolopale cell (Fig. 16). Moulins (1976) reviewed three different ways in which the attachment cell can attach to the cuticle or to epidermal cells.

1. In amphinematic scolopidia, the attachment cell surrounds the tube as an epidermal cell, and the tube inserts directly into the cuticle (Figs 16b, 17f). This is seen for Johnston’s organ, mouthpart chordotonal organs, collembolan tibio-tarsal and pretarsal chordotonal organs (Moulins, 1976; Crouau et al. 1987). In Diptera, Johnston’s organ may attach to an invaginated projection of cuticle (Uga and Kuwabara, 1965; McIver, 1985).

2. In mononematic scolopidia the attachment cell is still an epidermal cell, but it forms the connection between the cap and the cuticle. This is found in antennal chordotonal organs (Schmidt, 1974; Courbière-Tichané, 1975).

3. In mononematic scolopidia the attachment cell is subepidermal, and forms
a connection between the cap and one or more epidermal cells. This is the most common type of association between scolopidium and integument, and is found in tympanal organs, subgenual organs (Fig. 10b), tarsal organs, the tegula chordotonal organ (Fig. 5a), moth wing-hinge organ and antennal organs (Moulins, 1976; Kutsch et al. 1980; Yack and Roots, 1992).

4.5.1 Morphology

In the third category, the attachment cells are often cylindrical and elongate (e.g. Field, 1991). Since they can occupy a more internal position than seen in the first two cases above, the attachment cells may form an elastic ligament as seen in the tibial and femoral chordotonal organs of the leg (Figs 1a, 8), the locust tegula (Fig. 5a) and the moth wing-hinge organ (Kutsch et al. 1980; Matheson and Field, 1990; Yack and Roots, 1992). Functional elaboration is seen in the locust femoral chordotonal organ (Fig. 8), where the attachment cells form separate strands connecting groups of scolopidia to a long, thin cuticular apodeme and form the basis of physiological range fractionation in this organ (Section 3.5.3; Field, 1991; Shelton et al. 1992).

Different attachment cell morphologies occur in the tympanal and intermediate organs of the weta Hemideina (Ball and Field, 1981; Ball, 1981). Proximally, attachment cells are stellate with numerous branched projections into a hyaline matrix, while distally along the crista acustica they become lamellate and form parallel plates lying at an angle to the trachea upon which they rest. They are embedded also in a layered hyaline matrix which lacks a bounding membrane and which forms a gelatinous tent-like mass over the crista acustica.

4.5.2 Cytoplasm and junctions

The cytoplasm of attachment cells contains abundant microtubules which are usually oriented longitudinally (Moulins, 1976), but become irregularly oriented in stellate attachment cells (Ball, 1981). In some cases the microtubules are so densely packed that they occupy the entire cytoplasmic contents, particularly at the distal end (Field, 1991; Yack and Roots, 1992). Proximally, rough endoplasmic reticulum has been observed in an “onion body” form (Kutsch et al. 1980). The plasma membrane is often thrown into invaginated folds joined together by microtubule-associated desmosomes (Fig. 16a) (Toh and Yokohari, 1985). Septate desmosomes and spot desmosomes join adjacent attachment cells (Young, 1970; 1975; Hallberg, 1981; Yack and Roots, 1992; Devetak and Pabst, 1994). Contact with the cap is characterised by hemi-desmosomes often associated with microtubules clustered in dense fibrillar plaques (Yack and Roots, 1992), or clustered in solid hexagonal arrays (Füller and Ernst, 1973), around the cap. Hemi-desmosomes may occupy the circumference of the cap (e.g. Ball, 1981). Adjacent attachment cells and
adjacent epidermal cells are joined by desmosomes and septate junctions in both mononematic and amphinematic scolopidia (Hallberg, 1981). Distally, one attachment cell may contact several epidermal cells by apical and basal hemi-desmosomes (Moulins, 1976), or by branched distal extensions with desmosome junctions (mononematic scolopidia of the tegula; Kutsch et al. (1980). Epidermal cells contact the cuticle with hemi-desmosomes associated with microtubules (Fig. 16b). In Johnston’s organ of the sawfly, *Neodiprion sertifer*, the attachment cells form fibres that penetrate into the cuticle as projections from densely-packed microtubule arrays interspersed in heavy deposits of electron-dense material in the attachment cell (Fig. 16b, right side) (Hallberg, 1981).

4.5.3 The connective tissue sheath

The ligament formed by attachment cells is enveloped by a connective tissue sheath (neural lamella: Young, 1970). The sheath is composed of two layers: a thin, outer nonfibrous matrix and a thicker inner layer containing fibrillar material (Young, 1970; Field, 1991). The electron-dense fibrils do not show banding at high magnification (Yack and Roots, 1992). Proximally they are evenly distributed through the sheath, but distally they form into thick bundles surrounding individual attachment cells (Field, 1991) and stain red with acid fuchsin (Shelton et al. 1992). The sheath composition is unknown, although it is thought to contribute to the elasticity of the ligament.

5. Mechanics of the scolopidium

Foremost in problems facing chordotonal organ research is the question of how a mechanical stimulus is transformed by the scolopidial components into a depolarisation of the sensory neuron. Progress in various areas has led to a proliferation of hypotheses, but a combined approach of biophysical, ultrastructural and neurophysiological techniques is still lacking.

The recent successes in understanding the sensory mechanisms of vertebrate auditory hair cells exemplify a successful approach to this basic question in mechanoreceptor research (Howard et al. 1988). First, the ultrastructural foundation was established, then physical measurements of the mechanical compliance of the components were integrated with newly-discovered membrane properties, ion channels and electrical responses. In the case of scolopidia, the stage is set for considering mechanical compliances of various components, and of the scolopidia as a whole. We review the few papers that have touched on this, and then suggest a framework for further research. Also we review the current hypotheses for the action of scolopidia and recent work on membrane channels.
5.1 COMPLIANCE OF THE SCOLPIDIUM

Scolopidia are ultrastructurally unique due to the presence of an apparently robust mechanical reinforcement structure: the scolopale. Together with the cap (or tube) and the myriad of intercellular junctions between scolpidial components, the scolpidium appears to be a somewhat rigid apparatus, tightly held together. From the ultrastructure, the cilium, caged within the (rigid) scolopale, appears to be the key to activation of the sensory neuron. Given this construct, relative compliances of the different components must be assessed. Which might be stiff and which might bend easily? Which might be elastic and stretch; which might resist stretch? Where are the weak links? Scolopidia seem to fall into two groups based upon the above considerations: those with mononematic structure and those with amphinematic structure.

5.1.1 Mononematic scolpidium structure

5.1.1.1 Intercellular junctions  Mononematic scolopidia are solidly constructed by virtue of the great number of intercellular junctions joining nearly all of the components. Evidence for this conclusion is that living chordotonal organ cells ruptured rather than separated when slight pressure was applied to the organ under a cover slip in saline solution (Slifer and Sekhon, 1975).

The rationale for proposing a solid construction comes from the array of junctions summarised in Fig. 16a. Starting proximally, the scolopale cell is firmly attached to the dendritic inner segment by a belt desmosome which also has filamentous attachments to the ciliary root. The scolopale cell is attached to neighbouring scolopale cells by gap junctions and spot desmosomes, and distally to the attachment cell by septate desmosomes and microtubule-associated desmosomes. The scolopale cell fits “snugly” (Moran and Rowley, 1975) into the cap as it tightly encloses extensions of the scolopale rods which are inserted into the cap. If the cilium terminates in a bulb, it should have a firm placement within the cap (e.g. Toh and Yokohari, 1985).

The cap itself is anchored circumferentially to the overlying attachment cell by hemidesmosomes (Ball, 1981; Wolfrum, 1990; Yack and Roots, 1992), which suggests a solid attachment between these two structures.

The attachment cell is connected to neighbouring attachment cells by spot desmosomes and belt desmosomes. The presence of numerous invaginations of its plasma membrane bound together by microtubule-associated desmosomes suggests that attachment cells may have a heavily-reinforced bounding membrane (Moran and Rowley, 1975; Toh and Yokohari, 1985). Finally, attachment cells are anchored to epithelial cells by microtubule-associated desmosomes.

The following may be weak links in scolpidial ultrastructure. No junctions are described for the scolopale-to-cap attachment, which could either be a point of weakness or of relative longitudinal movement in the scolpidial
apparatus. The cilium of the dendrite inserts into the cap, but no specialised junctions have been described between the two structures. Sometimes the cilium fits snugly into the cap (e.g. Yack and Roots, 1992), but often there is space between the cilium and the lumen in the cap, even in cryofixed specimens which should show virtually no shrinkage artifacts (e.g. Wolfrum, 1990). It is questionable, then, whether the cilium is always tightly attached to the cap.

5.1.1.2 Cytoskeleton Scolopidia also appear to have solidity (stiffness?) conferred by elaborate cytoskeletal structures, in addition to solidity conferred by intercellular junctions. In contrast to the soma portion of the sensory neuron, which is not particularly well endowed with cytoskeletal structures, the dendritic inner segment contains axial microtubules throughout its length (Ball, 1981; Toh and Yokohari, 1985). A cytoskeletal meshwork for the dendrite is thought to be formed by the combination of microtubules connected to the ciliary roots by lateral filaments (Moran and Rowley, 1974). The $9 \times 2 + 0$ axoneme provides the cilium with a cytoskeleton in which the microtubule pairs are joined together by (presumably) dynein arms. The axoneme is apparently strong enough to induce active mechanical distortion of the ciliary base during stimulation (Moran et al. 1977). However, the disruption and expansion of the axoneme at the ciliary dilation should yield a point of weakness in the cilium since it would be the site of greatest strain (Howse, 1968; Slifer and Sekhon, 1975). Therefore it is necessary to know if the dilation alters the mechanical integrity of mononematic cilia.

The scolopale cell contains a rich microtubular cytoskeleton in the proximal, non-vacuolated region (Ball, 1981; Devetak and Pabst, 1994). In the vacuolated region the extensive labyrinth provides the cell with a profusely-interconnected spongy matrix of plasma membrane but a cytoskeletal meshwork is absent. The scolopale rods form a massive cytoskeletal system in that part of the scolopale cell surrounding the scolopale space. Within the scolopale, microtubules are surrounded by a framework of actin filaments and tropomyosin, which (Wolfrum concludes) make the scolopale both a stabilising and an elastic structure (Wolfrum, 1991a,c).

Scolopales in different chordotonal organs are likely to differ in strength and in supporting function, inasmuch as some are incomplete, non-cylindrical structures (see Section 4.4.3). Empirical tests of scolopale stiffness and longitudinal elasticity are required. These might be carried out using fine calibrated glass rods, as used for motile cilia stiffness measurements (Okuno and Hiramoto, 1979). It is likely that the scolopale rods are under lateral tension, for Slifer and Sekhon (1975) noted that the proximal ends of the rods spring apart when a living chordotonal organ is ruptured under a coverslip.

Attachment cells have the highest concentrations of axially-oriented microtubules of any cell in the scolopidium. Many microtubules are attached to apical and basal desmosomes and become more densely packed distally. Since microtubules are relatively inelastic (McIntosh and McDonald, 1989) it
would seem that these cells are designed to efficiently transmit force from the cuticle to the cap. An exception to this conclusion is discussed in Section 5.1.4.

In summary, mononematic scolopidia appear to be greatly strengthened by intercellular junctions and elaborate cytoskeletal structures, except for three potentially weak points: the junction of the scolopale rod to the cap, the junction of the cilium to the cap, and the ciliary dilation.

5.1.2 Amphinematic scolopidium structure

Less information is available on the intercellular junctions and cytoskeletal structures of amphinematic scolopidia. Most descriptions apply to scolopidia of Johnston’s organ.

5.1.2.1 Intercellular junctions Fewer junctions appear to cement the components longitudinally in amphinematic scolopidia, and several points could serve as weak links (Fig. 16b). Examination of scolopidia, beginning at the proximal end, reveals the following junctions (details in Section 4.5.2). The belt desmosome between the dendrites and scolopale cell in amphinematic scolopidia is similar to that of mononematic scolopidia. The scolopale cell is joined to the attachment cell by septate desmosomes (the dense desmosomes of mononematic scolopidia are lacking), and the attachment cell joins the cuticle directly by means of microtubule-associated hemi-desmosomes and/or penetrating electron-dense fibres. Lateral connections between components include symmetric desmosomes that join adjacent dendritic inner segments (Moulins, 1976), longitudinal gap junctions and layered desmosomes that join adjacent cilia in Type 2 scolopidia (Courbière-Tichané, 1971; 1975), and septate and belt desmosomes that join neighbouring attachment cells. Neighboring scolopale cells lack lateral junctions, in contrast to mononematic scolopidia.

Weak links in the structure appear to be in the junction between scolopale cell and attachment cell, and the discontinuous link between the tube and the scolopale (compare Fig. 16a,b). Also the scolopale cell and attachment cell do not make special connections to the tube, although they may closely appose it (Fig. 9.5b in Moulins, 1976).

5.1.2.2 Cytoskeleton The cytoskeletal enhancements seen in mononematic scolopidia are not as plentiful in amphinematic scolopidia, and there are some basic differences in the structure of the cytoskeleton within the cilium.

Few microtubules are found in the dendritic inner segment (Courbière-Tichané, 1971, 1975). The ciliary root is often short or non-existent in Type 2 cilia. In the cilium itself, a dilation is usually absent, and in Type 2 cilia the axoneme is lost midway and a microtubule-filled extension projects into the tube. This often forms a tubular body at the termination of the cilium, which
may be embedded in the cuticle or arthrodial membrane; such a structure is never seen in mononemetic scolopidia.

The scolopale cell contains abundant microtubules, but the labyrinth is not as extensively developed as in mononemetic scolopidia (Courbière-Tichané, 1971, 1975; Vande Berg, 1971). The scolopale is often thinner and longer than those of mononemetic scolopidia, and distally it is not strongly anchored to the tube. It does not appear to provide a strong barrel-like support structure as seen in mononemetic scolopidia, where it is inserted into the cap (Fig. 16a,b).

Attachment cells and epidermal cells also contain abundant microtubules.

In summary, amphinemetic scolopidia have strongly joined and internally reinforced distal structures, but proximally there are several areas of weakness. Specifically, the junction between the scolopale cell and the attachment cell may be weaker than in mononemetic scolopidia and neither connects to the tube, the scolopale itself is not connected to the tube, the cilia appear to slide within the tube, and the ciliary roots do not seem to be strong anchoring structures. Further, the dendritic inner segments are not well reinforced by microtubules. A major difference from mononemetic scolopidia is that the tips of the cilia could be laterally compressed if they are embedded in cuticle, as in Johnston’s organ.

5.1.3 Avenues for experimentation

In order to understand how these cellular and intracellular components react to an applied stretch or relaxation stimulus, it is necessary to know their relative elasticities (Wolfrum, 1990), and the strengths of connections between components. The structure of mononemetic scolopidia is considered here. Based upon densities of microtubules and solid scolopidial material, the stiffest (least elastic) components appear to be the attachment cell, the cap, the scolopale and the ciliary roots (Toh and Yokohari, 1985). The two strongest inter-component connections appear to be the connection of the attachment cell to the cap and scolopale cell, and the connection of scolopale cell and rods to the dendritic inner segment via the collar. This scenario seems to place the cilium in a mechanically protected position, and to transmit the stimulus force directly to the dendritic inner segment through the scolopale rods. However, many authors (see Section 5.2) have felt that the cilium is unlikely to be excluded from action in such an elaborate histological construction.

The involvement of the cilium would differ depending upon whether the direction of pull on the scolopidium is axial or off-axis. The elasticity (Young’s modulus: stress/strain) of microtubules in motile cilia has been estimated to be 3x10^9 N/m^2; this is approximately the stiffness of external tibial cuticle and twenty times the stiffness of muscle apodeme in the locust Schistocerca (Hannah and Hillier, 1971; Ker, 1977; Okuno and Hiramoto, 1979). Since both microtubules and actin (which is presumed to have a similar Young’s modulus to that of microtubules; Howard and Ashmore, 1986), make up scolopale rods,
it could be expected that scolopale rods show a greater resistance than cilia to axial pull, in proportion to the greater cross-sectional area of the rods. Since the rods are in parallel with the cilium in mononematic scolopidia, it follows that the cilium cannot be stretched unless (a) the cap can slide distally relative to the rods due to a weak rod to cap linkage, or (b) the cap becomes elongated under axial pull, or (c) the ciliary roots contract. Therefore, if axial pull is to be considered the effective stimulus, experiments must focus on these scolopidial properties.

If off-axis pull is the effective stimulus, then it is necessary to assess the degree to which (a) the cap can be tilted relative to the axis of the scolopale, or (b) the rods can buckle. In either case the action must transmit some bending shear to the cilium. At least one major hypothesis considered below requires such shear (Section 5.2).

Clearly amphinematic scolopidia do not have the same structural design. Because they have a tubular body at the terminus of the cilium or ciliary derivative, Thurm (1965) and Moulins (1976) suggested that these scolopidia are designed to respond to lateral compression of the tubular body with local opening of ion channels. The cilium would then serve to conduct the electrical signal proximally to the dendritic inner segment. However not all such cilia terminate in cuticle as seen in Johnston’s organ, and the crucial question is whether these cilia are free to slide within the tube, or are held and stretched by the relatively inelastic tube and attachment cells.

The strength of junctions between components remains to be investigated, perhaps beginning with measurement of the total areas of each type of junction. The degree to which various junctions yield to stretch could also give information about relative strengths.

### 5.1.4 Elasticity of whole chordotonal organs

Despite the above arguments for stiff, inelastic elements in scolopidia, some chordotonal organs, such as the femoral chordotonal organ in the leg, are highly elastic and undergo significant elongation and return during normal leg movements. In collaboration with P. Shelton, R.O. Stephen and M. Walker, one of us (LHF) has demonstrated that the FeCO behaves as a viscoelastic body, the ligament of which obeys Hooke’s Law ($F = -kx$), where the force resisting stretch, $F$, varies with displacement, $x$, by the proportionality constant, $k$. This relationship holds within an elastic limit. As in other viscoelastic materials, cyclic stretch and relaxation cause hysteresis in resistance, and upon sudden step increases in length, the ligament shows creep as elasticity overcomes viscosity. These properties are likely to have a significant bearing upon the response properties of the sensory neurons in the FeCO, and they are most likely centred in the attachment cells which compose the ligament. In view of the assumed highly inelastic nature of the attachment cells with densely packed microtubules, it becomes crucial to establish whether the microtubules
slide past each other during stretch, and if so, how recovery is regulated. These questions are currently being investigated.

5.2 HYPOTHESES FOR ROLE OF CILIA IN MECHANICAL COUPLING

French (1992) defined three steps for the process of mechanotransduction in animals: coupling of the mechanical stimulus to the sensory neuron, transduction of neuronal membrane deformation into a receptor potential and, finally, coding of the receptor potential into the firing frequency of spikes. In insect chordotonal organs, the cilium is implicated in the first, and in some cases the second, of these steps. Current models propose that the cilium receives mechanical distortion either (a) through stretch applied axially (or slightly obliquely), or (b) through lateral compression applied to the apical tubular body (Moulins, 1976; McIver, 1985).

5.2.1 Axial stretch

5.2.1.1 Distortion of the ciliary dilation  Howse (1968) proposed that the ciliary dilation would be most susceptible to axial stretch because the axonemal microtubules here are more separated than elsewhere along the cilium. This hypothesis assumes that the axoneme acts as an internal cytoskeleton that resists change in diameter. Stretch of the cilium would be induced by flexion between the cap and the scolopale rods in mononematic cilia. Moran and Rowley (1975) proposed that stretch could cause distal sliding of the cap along its finger-like insertions of the scolopale rods. This, in turn, would cause stretch of the cilium at the dilation due to (a) lack of reinforcing dynein side arms on the A-subfibres of the dilation axoneme, and (b) the existence of crossbridges that connect the dilation membrane to the axoneme microtubule doublets. Thus stretch of the dilation would yield a relative difference in movement of membrane and axoneme, resulting in the development of shear forces in the membrane. This could lead to opening of ion channels in the dilation membrane and subsequent depolarisation of the dendrite.

5.2.1.2 Pull transmitted along a stiff cilium to a dendrite  Assuming that the scolopale rods slide along their insertions into the cap, axial displacement of the cap could pull on the cilium, which, because of its stiffness, could transmit the pull directly to the dendritic inner segment (Young, 1970; Toh and Yokohari, 1985). The terminal bulb seen in some cilia (Toh and Yokohari, 1985) would ensure that the cilium is firmly anchored as the cap pulls it. In this scheme, Young (1970) ruled out the possibility of flexion of the cap with respect to the scolopale rods inasmuch as the latter insert a long way into the cap. Moran and Rowley (1975) suggested a similar alternative for the SGO, but concluded that the whole cap+scolopale+cilium apparatus was so stiff that it would transmit mechanical distortion directly to the balloon-like inflation of
Fig. 20. Possible roles of cilia in mechanical coupling of stimulus to neurone. 

**a.** Model in which off-axis pull on the cap produces active sliding and bending of the axoneme. 

**b.** Evidence underlying a helical model of ciliary contraction, drawn from crustacean and insect material. 

**i.** Transverse section of antennal chordotonal cillum from the crustacean *Antromysis juberthiei* (Mysidacea). The structures associated with the membrane appear as paired protruberances (arrowheads); bridges join certain protruberances at the outer arms of the doublets (arrows). 

**ii.** Transverse section of cillum from Johnson’s organ of the beetle *Speonomus pyrenaeus* showing the unusual thickness of the ciliary membrane. The inner face is covered with osmophilic material; bridges link outer arms of the doublets to this material (arrows). 

**iii-v.** Details of cilia from *S. pyrenaeus* (iii), *Atyaephyra desmarestii* (a freshwater shrimp, Atyidae) (iv), and *Nyphargus longicaudus* (a gammarid isopod) (v). Individual protruberances (arrowheads) appear on the inner face of the ciliary membrane of all three preparations. Bridges link some of the protruberances to the outer arms of certain microtubule doublets (arrows). **vi-vii.** Diagrammatic comparison of the structure of 9+0 type cilia in crustacean and insect.
the dendritic inner segment just proximal to the scolopale rods. This inflated section of the
dendrite (Fig. 12b, described in Section 4.3.1) lacks microtubular support and is thought to
have the greatest compliance in SGO mononematic scolopidia (Moran and Rowley, 1975).

5.2.1.3 Active or passive bending of the cilium The possibility of active stroke propagation
along the cilium was raised by Moran et al. (1975). A slight bend in the tip of the cilium caused
by the mechanical stimulus was thought to induce an active stroke in the cilium in the region
containing dynein (identity not proven) arms on the axoneme A-tubules. The bend would be
propagated to the base of the cilium where the dendritic membrane would be distorted, leading
to ionic conductance increase.

However, because sensory cilia lack some of the components found in motile cilia (reviewed in
McIver, 1985), Moran et al. (1977) modified the hypothesis to propose a stretch-induced active
sliding of the axonemal microtubules. This is based upon ultrastructural evidence showing a
bend in the proximal section of cilia that were fixed during maximal stretch stimulation. The
scheme applies to the femoral chordotonal organ (proximal scoloparium) in which
mononematic scolopidia lack the inflated inner dendritic segment. The proposed action (Fig.
20a) is as follows: (a) oblique (off-axis) pull slightly displaces the tip of the cilium laterally, (b)
lateral tip displacement induces active sliding of adjacent axoneme doublets based upon action
of the dynein arms, (c) the bend is transferred to the distal basal body at the base of the cilium,
(d) bending of the ciliary base distorts the membrane in the region of

Fig. 20 caption continued.

chordotonal organs. vi. Protruberances line the inner face of the ciliary membrane in A. juberthiei. vii. In
the insect and the other two crustaceans, the protruberances are buried in the osmophilic material lining
the membrane inner face. Scales (in µm): i, 0.1; ii,iii, 0.07; iv, 0.12; v, 0.1. a, Modified from Moran et
al. (1977), with permission; b, from Crouau (1983), with permission.
the ciliary necklace (see Section 4.3.3.6.1), and (e) membrane distortion causes local changes in ion conductance leading to the production of a generator potential. The theoretical basis of the production and propagation of the bend in the cilium is based upon the application of catastrophe theory, which predicts that a small displacement of the tip of the cilium will lead to a considerable bend at the base of the cilium (Varela et al. 1977).

The hypothesis is still problematic and requires further testing. It is not clear, for example, how the bend could be transferred through the ciliary dilation, which lacks dynein arms, nor what action results from the attachments between the dilation membrane and the axoneme. In addition, many scolopidia are not positioned at an angle to the direction of stretch (taken to be the longitudinal axis of the attachment cell). Even if scolopidia lie at an angle to the direction of pull (e.g. Young, 1970; Moran and Rowley, 1975; Zill, 1985a) there is no proof that the pull at the distal end of the attachment cell is oriented in the same direction as that at the proximal end. For example, long attachment cells, such as those in the FeCO, curve as they progress proximally from the narrow distal attachment site to the broad expanse of the chordotonal organ (Field, 1991). A study is in progress to resolve this problem by examining the actual movement of scolopidia in fluorescently-labeled cells during pull of the chordotonal organ (M. Walker, pers. comm.).

5.2.1.4 Helical contraction of a cilium The inner surface of the ciliary membrane contains tiny protruberances which appear to form crossbridges with the outer arm of the A-tubule pair of dynein arms, and with the shared area of the A and B-tubules (Fig. 20b). Assuming that the crossbridges and the dynein arms operate in a make-break fashion, Crouau (1983) proposed that a cyclic succession of bridging between the microtubule doublets and membrane protruberances would result in a rotation of the membrane relative to the axoneme. Due to the two different sites and directions of bridging, the cilium would twist into a helicoidal form. This would develop membrane strain at the cilium base and lead to ion conductance changes and development of the generator potential.

5.2.1.5 Inertial drag during vibration The mononematic scolopidia of the antennal connective chordotonal organ are sensitive to vibrational stimuli (Toh and Yokohari, 1985). To account for such sensitivity, these authors note that the attachment cell, scolopale cell and dendrite are firmly bound together by desmosomes, and are invested with abundant microtubules. As a result, the assembly should vibrate as a single rigid mass. However the cilium is free in the scolopale space, being anchored only at either end. During vibration, there could be a point at which the cilium fails to follow the displacement of the dendrite. With increasing frequency of vibration, the inertia of the cilium should cause it to undergo a waving or bending motion. If the cilium resists the deformation due to internal microtubule stiffness, it may transmit strain on
to the dendritic inner segment and thus alter local ion conductances to cause depolarisation. Inherent in this hypothesis is the unproven assumption that, to establish a waving motion, there must be a lateral motion vector in the vibrational stimulus. In addition it is necessary to determine whether the ciliary dilation causes a compliance disruption in the cilium stiffness and interferes with the proposed waving motion.

5.2.1.6 Bending of scolopale rods  Immunohistochemical studies have shown that scolopales contain actin and tropomyosin (Wolfrum, 1990, 1991c; Section 4.4.3). Actin could allow bending, but presumably not extension, of scolopales during oblique pull, and provide a restoring force to bring scolopales back to their pre-stimulus shape (Wolfrum, 1990, 1991a,b,c). Tropomyosin is a calcium sensitive protein known to bind to actin and to enhance filamentous actin rigidity (Wolfrum, 1991c). Hence it is possible that any lateral vector of a mechanical stimulus could cause bending of the scolopale rods and concomitant modification of the existing tension on the cilium between its proximal and distal attachments. Wolfrum (1990) proposed that this action could accompany any of the above hypothesised transduction mechanisms, and that its sensitivity could be modified by altered internal Ca\(^{2+}\) concentration.

5.2.1.7 Contraction of ciliary rootlets  Based on demonstration of a centrin-like protein in the ciliary rootlets, Wolfrum (1991b) proposed that the ciliary rootlets might undergo contraction in the presence of raised Ca\(^{2+}\) levels, in a manner similar to that seen in the centrin-containing rootlets of green flagellate algae (Höhfeld et al. 1988). The cilium could experience either an increased longitudinal tension if the effective stimulus is axial pull on the scolopidium, or a shift in position of the distal basal body, to enhance bending in the ciliary necklace region if an active ciliary stroke is the effective stimulus. Furthermore, Wolfrum argued that relaxation of the ciliary roots (resulting from decreased cytosolic Ca\(^{2+}\) concentration) could be a mechanism for sensory adaptation of the neuron through decreased tension on the cilium.

5.2.2 Lateral compression

5.2.2.1 Mononematic scolopidia  Thurm (1965) showed that the dense tubular body at the tip of the modified cilium of insect hair-plate sensilla is the site of lateral compression during bending of the hair. In a like fashion, he proposed that the conical cap enclosing the cilium terminal should elongate, become narrower and compress the cilium during axial stretch of a scolopidium. Such a transformation of mechanical energy could lead to a considerable increase in the effective stimulus acting on the cilium through amplification of longitudinal pull into lateral compression. This could explain the difference between the threshold vibrational amplitude observed for
chordotonal receptors (around 0.01 nm, but see Shaw, 1994b) and the threshold compression required for the tubular body in the hair-plate sensillum (around 1 nm), assuming both used the same mechanism for generation of the receptor potential through membrane distortion (Thurm, 1965). Though not tested for scolopidia, the idea could be modelled if the nature of the cap material were elucidated. Validity would hinge upon the surface distribution of force applied to the cap by the attachment cell, as well as the compressibility (elasticity) of the cap material.

5.2.2.2 Amphinematic scolopidia A mechanism for lateral compression of the ciliary tip is more plausible in the amphinematic scolopidia of Johnston’s organ (Fig. 11b). Here the tip of the cilium (often containing a dense tubular body), encased in the tube, can be embedded in the flexible cuticle of the joint between the antennal pedicel and the flagellum (e.g. Hallberg, 1981). Angular movement of the flagellum about its insertion could cause lateral compression of the cilium (Thurm, 1965; Moulins, 1976). The detailed ultrastructural specialisations between the ciliary membrane and the microtubules of the tubular body in insect hair sensilla (Thurm et al. 1983) have not been sought in the tubular body of amphinematic cilia; confirmation of a similarity of structure would have implications for homologies between sensilla containing tubular bodies.

6. Transduction mechanisms

It is generally accepted that direct mechanical distortion of the dendritic membrane in mechanosensory receptors generates an ionic conductance. The resultant current creates a receptor potential across the membrane which is graded in proportion to the stimulus intensity (French, 1988). In insects, this model is based upon work carried out on various hair and campaniform sensilla, most of which has been reviewed by Schwartzkopf (1974), McIver (1985) and French (1988, 1992). Little is known about equivalent mechanisms in chordotonal sensilla.

6.1 Mechanically activated channels (MACs)

Widespread evidence from a variety of animal (and some plant) tissues suggests that stretch-sensitive or mechanically-activated channels (MACs) in the dendritic membrane mediate the ionic transduction current (Erxleben et al. 1991; French, 1992). Most MACs are permeable to a range of monovalent cations (i.e. they are nonselective cation channels), although some are selective for potassium only and others are permeable to divalent as well as monovalent cations. Simultaneous anion and cation permeability of single channels also occurs (French, 1992).
Universally, membrane tension affects the probability of the channel being open, rather than the channel conductance. The channels thus conform to the expected gating behaviour with one or more tension-dependent states, rather than representing tension-altered holes in the membrane (French, 1992).

Although MACs represent an attractive mechanism for conductance changes in mechanoreceptors, they have been found in only two arthropod examples, one of which is a Type I receptor and the other a Type II receptor. In the former, cation-permeable MACs were found, but not extensively described, in cultured scolopidial neurons isolated from the cockroach antennal connective chordotonal organ (Stockbridge and French, 1989). The neurons had lost their scolopale and distal dendritic segment, but were apparently healthy. The contained a stretch-activated channel permeable to both potassium and sodium with a conductance of about 100 pS (Stockbridge and French, 1989). The same cultured neurons possessed two mechanically insensitive potassium channels and a chloride channel (Stockbridge et al. 1990).

The Type II receptor (crayfish MRO) MACs included two types of monovalent and divalent cation-permeable channels on the soma and dendrites (Erxleben, 1989). One type, the stretch-activated (SA) channel, was voltage-insensitive and permeable to potassium (71 pS), sodium (50 pS) and calcium (23 pS); it was deemed to mediate the transduction current. The other type showed strong inward rectification (RSA channel), but had the same non-selective cation permeabilities as the SA channels. Its function is unclear. A calcium permeable MAC mediates the potassium-induced rapid phase of adaptation in the stretch receptor neuron (Erxleben, 1993).

In the examples above, the patch-clamp samples containing MACs were probably not from the mechanotransduction regions, which raises the possibility that the channels have different functions (French, 1992). For studies of MACs in chordotonal neurons, access to the transduction regions of the cells is required. If such regions are restricted to the ciliary membrane or terminal aspect of the proximal dendritic segment, direct patch-clamp recordings will probably be impossible in intact scolopidia, but it may be possible to explant such membrane regions to host cells or to artificial membranes for study. Extensive details for recording from stretch-activated channels are provided by Erxleben et al. (1991).

6.2 Receptor Currents and Potentials

In insect cuticular sensilla (but not chordotonal sensilla), the ionic milieu of the dendritic outer segment is very different from that of the haemolymph (reviewed by McIver, 1985; French, 1988). The surrounding tormogen (sheath) cells create a high potassium concentration around the cilium by pumping potassium ions into the receptor lymph space enclosing the cilium. In addition,
tight junctions between the sheath cells create a high electrical resistance between the receptor lymph space and the haemolymph. As a result, the cilium faces a rather small potassium concentration gradient but experiences a large transmembrane potential of about 140 mV (Thurm and Wessel, 1979). This could favour a strong inward receptor current carried by potassium in response to distortion of the membrane of the tubular body in cuticular sensilla. Under such conditions a regenerative spike potential of unusual amplitude and waveform should occur in the apex of the cilium (Erler and Thurm, 1981). Analogous ionic compartments and voltages could occur in chordotonal sensilla. A similar receptor lymph space (scolopale lumen) surrounds the cilium. The mesaxonal and basal laminated junctions of the scolopale cell could electrically isolate the scolopale lumen from the haemolymph.

Evidence in chordotonal organs for mechanisms analogous to those of hair sensilla have been demonstrated in insect auditory sensilla (Hill, 1983a,b; Oldfield and Hill, 1986). Intracellular recordings were obtained from various parts of the sensory neurons and attachment cells of Müller’s (tympanal) organ in the locust and of the crista acustica in a tettigoniid (*Caedicia simplex*). With the recording electrode placed in the dendrite, Hill (1983a) discovered that the first electrical sign of mechanosensory transduction comprises a train of discrete subthreshold depolarisations of variable amplitude up to about 5 mV (Fig. 21a), rather than a slow, smoothly graded receptor potential, and these were likened to quantum bumps found in photoreceptors. A noise analysis indicated that the elementary event is 0.19 mV in amplitude, with a first-order exponential time constant of 5-10 ms. The discrete depolarisations of the auditory receptors are relatively voltage-independent and appear to be driven toward an equilibrium potential that is positive with respect to the resting potential. They occur (a) spontaneously at random in the absence of sound, and (b) with increasing probability of occurrence and number of depolarisations as sound intensity increases (Hill, 1983a). They summate to produce a graded receptor potential which shows time-dependent adaptation and is characteristically noisy (Fig. 21b).

Hill (1983a) proposed a scheme in which mechanical energy is absorbed by specialised molecules tentatively located in the cilium. Activation would lead to chemical signalling of the conductance channels in the dendritic plasma membrane via an internal messenger. This is based upon analogy to a similar mechanism proposed in visual systems, where the internal messenger would provide the necessary amplification from the absorption of a single photon to the opening of sufficient conduction channels to yield a single quantum bump potential. Adaptation could occur in both systems as a result of depletion of the proposed internal messenger.

Both the discrete transduction potentials of the chordotonal neuron and the quantum bump depolarisation in *Limulus* photoreceptors show an exponential decay (Hill, 1983a). Thus the chordotonal neuron transduction process could be usefully modelled as a series of exponential relaxation processes, as
carried out by Thorsen and Biedermann-Thorsen (1974) for the transduction dynamics in Limulus. The relaxation model utilised fractional power-law dynamics.

6.3 TRANSDUCER COUPLING TO SPIKE GENERATOR

6.3.1 Evidence for dendritic spiking

Hill (1983b) showed that the receptor potential causes non-propagating spikes believed to arise in the apical region of the inner dendritic segment (Fig. 21b,c). These are unusual in that they have small amplitudes (about 25 mV in neurons with resting potentials of about 60 mV), and do not undershoot the resting potential on the repolarizing phase. They are thought to be generated in the distal dendritic membrane and to result from unusual ionic concentrations in the scolopale space. They appear to be electrotonically conducted to the basal region of the inner dendritic segment, where they always precede, and trigger, an action potential driven by conventional Na⁺ and K⁺ conductances (the basal dendritic spike, Fig. 21c). Basal dendritic spikes in turn depolarise the axonal trigger zone to produce orthodromically conducted axonal spikes (Fig. 21c).

Oldfield and Hill (1986) subsequently pinpointed the site of the apical spike by recording simultaneously from an attachment cell and chordotonal neuron in the tettigoniid crista acustica (Fig. 21d). By observing the effects of current injection into the attachment cell, and retrograde invasion of axonally-stimulated spikes, they confirmed that the small apical spikes are indeed derived from the apex of the inner dendritic segment, which is coupled resistively to the attachment cell through the scolopale lumen.

Thus it appears that in chordotonal sensilla, as in cuticular sensilla (Erler and Thurm, 1981), the elongated dendrite has an apical spiking process, driven by unusual ionic gradients associated with the receptor lymph, which serves to amplify the sensory signal derived from the distal aspect of the sensillum and ensure firing of the neuron’s spike-generating zone. The scolopale lumen clearly invites investigation, perhaps with the aid of X-ray microprobe technology used in scanning electron microscopy.

6.3.2 Coding of spike frequency

Electrical coupling between the transducer region and the trigger zone of mechanosensory neurons is usually subject to some form of adaptation which is reflected in the spike discharge. Adaptation can be due to time-dependant processes in both regions of the neuron. For example, the transducer region can experience mechanical uncoupling, as seen in the classic textbook examples of the Pacinian corpuscle and the rapidly-adapting crayfish abdominal stretch receptor (Aidley, 1989; Mellon, 1968). The responsiveness of the trigger zone...
Fig. 21. Electrical events associated with response of tympanal scolopidium to sound. a. Discrete depolarisations of a locust tympanal neuron evoked by a 4 kHz tone (bar). b. When sound stimulus intensity (lower trace) is increased in 10 dB steps the discrete potentials sum to produce a sustained, graded receptor potential which leads to spikes. Adaptation is more prominent at lower intensities. c. Scheme proposed by Hill (1983b) for the sequence of excitatory events in a stimulated neuron. Subthreshold discrete potentials, thought to arise at the cilium (outer dendritic segment), lead to a graded receptor potential and small, non-propagated apical spikes in the apical dendritic membrane. Apical spikes passively invade the basal region of the dendrite and trigger larger basal dendritic spikes which propagate to the soma and axon where they trigger axonal spikes. The latter two are conventional action potentials, whereas the apical spikes never undershoot and are thought to involve different ionic conductances associated with the receptor lymph space of the scolopale cell. The attachment cell is truncated. d. The attachment cell of a bush cricket tympanal scolopidium shows negative spike potentials corresponding with apical spikes recorded simultaneously from its associated sensory neuron. Both cells were filled with Lucifer Yellow after recording. The stimulus is a 14 kHz tone. Scale: 50 µm. Calibrations: a. 40 ms, 2 mV; b. 100 ms, 20 mV; d. 10 ms, 5 mV (upper trace), 40mV (lower trace). a, Modified from Hill (1983a), with permission; b,c, Modified from Hill (1983b), with permission, d, modified from K. Hill and B. Oldfield (unpublished), with permission.
to receptor currents can change with differences in rate of rise of subthreshold responses (accommodation) as well as variation in spike threshold over time (accumulated refractoriness). In tonic units these processes reach a steady state following adaptation and the plateau spike frequency is linearly related to amplitude of the receptor potential. The logarithmic relationship of spike frequency to stimulus intensity is therefore considered to be a function of the transducer region (Mellon, 1968; Mill and Price, 1976; Aidley, 1989).

To what extent have similar coding properties been uncovered in chordotonal neurons? Although relevant data are unquantified, some conclusions may be reached from inspection of intracellular soma recordings of Müller’s organ auditory neurons and FeCO neurons. During constant-intensity sound stimulation, graded subthreshold receptor potentials show adaptation within 100ms which is independent of membrane potential, and therefore appears to represent a decrease in number of available conductance channels (Hill, 1983a). Other neurons lack adaptation, suggesting that the transducer region in neurons of the locust ear shows various degrees of adaptation. Analysis of Hill’s published records shows that the relationship between receptor potential and sound intensity is not logarithmic, but instead is approximately linear. Constant current injection through microelectrodes into locust FeCO somata produces spiking adaptation at the trigger zone within 20 s (Zill, 1985a). A greater degree of adaptation occurs when the same neuron responds to joint displacement that gives a similar level of initial firing. For this particular tonic neuron, the additional rate of adaptation is attributed to the transducer region.

In summary, some scolopidial neurons show adaptation in the transducer region and in the trigger zone, whereas others may show no adaptation. The relationship between stimulus intensity and receptor potential amplitude is not necessarily logarithmic. The relationship between receptor potential amplitude and spike frequency of the trigger zone has not been estimated. Quantitative studies of soma and dendrite recordings would help to establish basic dynamics and differences between scolopidial neurons of various chordotonal organs, and would therefore allow an estimation of the
contribution of electrical membrane components to chordotonal neuron responses.

Discrete subthreshold depolarisation events such as those observed in tympanal organs have also been found in tonically-spiking neurons of the femoral chordotonal organ (FeCO), where they summate and give rise to overshooting spikes (Field and Matheson, unpublished observations). In the subgenual organ (SGO) of the cockroach, a slow receptor potential gives rise to non-overshooting spikes, but shows no sign of discrete subthreshold events. The recording site was in an electrotonically coupled accessory cell, which may explain the absence of discrete depolarisations (Shaw, 1994b). Therefore it is not clear whether Hill’s (1983a) results for tympanal scolopidia can be generalised to other scolopidial receptor potentials.

The transduction region of chordotonal sensilla is much more susceptible to blockage by dimethyl sulfoxide (DMSO) than the axonal region (Theophilidis and Kravari, 1994). DMSO has an analgesic effect on vertebrate sensory neurons but the mechanism of action is unknown. In the locust FeCO, evidence suggests that potassium transduction channels in the dendrite are blocked at low concentrations (0.85%) and axonal potassium conduction channels are blocked at higher concentrations (4.8% reduces the firing rate by half; Theophilidis and Kravari, 1994).

7. Physiological responses of chordotonal organs

7.1 Mechanosensitive roles of chordotonal organs

In an ecological approach to insect mechanoreception, Schwartzkopf (1974) summarised the senses that insects use to detect mechanical signals in the environment. When focused on chordotonal organs specifically, the scheme gives an appreciation of their versatility.

A gravitational sense is achieved by evaluation of displacement of body parts as an insect rests in, or alters its position in, the gravitational field (a constant mechanical force). Leg joint chordotonal organs detect errors caused by gravitational pull, in a feedback loop set to maintain posture (Bässler, 1993; Field and Coles, 1994), whereas antennae in resting dipterans signal the direction of the gravitational field (Schneider, 1953).

A sense of air or water current is given by Johnston’s organ, and by hair sensilla, in detecting displacement of the surrounding medium relative to the insect’s body. If the insect is resting on the substrate, deflection of the antennae by moving air or water causes orientation into the current. In flying insects, the Johnston’s organ, and in some cases campaniform and hair sensilla, detect antennal deflection in a feedback system that controls wing beat rate, and therefore flight speed, to achieve a stable airflow against the antennae (reviewed in Schwartzkopf, 1974).
A vibration sense (oscillations of a solid substrate, as opposed to air or water) is highly developed in insects, owing to the SGO in each leg. Usually the SGO scolopidia respond best to low frequencies up to several kHz and over comparatively short distances (because of damping by the substrate).

A sense of hearing, on the other hand, involves detection of oscillations in a fluid (air or water) over long distances. Both vibration detection and hearing are important in communication amongst insects. As seen in Section 3, insects have a great diversity of chordotonal organs and associated structures adapted for hearing, allowing acoustic sensitivity peaks ranging from 600 Hz to 200 kHz (see Table II compiled by Schwartzkopf, 1974). The range includes ‘primitive’ hearing organs, such as tarsal chordotonal organs of backswimmers (Notonectidae) which detect water surface oscillations as weak as 0.5 µm at 150 Hz, and ‘multifunctional’ chordotonal organs, such as Johnston’s organ, which detects not only wind current but also the specific sound frequency of female wing beats in culicid dipterans (380 Hz for the mosquito Anopheles subpictus). Hearing organs have developed in mouthparts (sphingid moths) and wings (lacewings, Neuroptera) for specific detection of high frequency airborne sound, albeit without elaborate tympana. The most specialised hearing organs are the tympanal organs, with elaborate modifications for high sensitivity, and frequency tuning of individual scolopidia (Schwartzkopf, 1974; Michelsen and Larsen, 1985).

There are two senses in which insect chordotonal organs do not appear to participate. The sense of touch is by definition the domain of cuticular sensilla and campaniform sensilla. The sense of isometric tension within and between internal body parts, especially associated with muscles, appears to be restricted to Type II multipolar receptors (e.g. the tibial flexor muscle tension receptors in locusts: Theophilidis and Burns, 1979; Matheson and Field, 1995), although in crustaceans, chordotonal organs can detect muscle tension as well as displacement of joints (Macmillan et al. 1982).

7.2 The Nature of the Stimulus

The ultrastructure of scolopidia indicates that these sensilla are designed to be exquisitely sensitive bio-mechanical transducers, but it does not suggest the kind mechanical signals to which scolopidia respond. Mechanical energy occurs in a variety of forms, including static potential energy involved in position and force (shear, torsion, compression, stretch) and kinetic energy involved in displacement (direction, velocity and acceleration). When this energy impinges on the rigid arthropod exoskeleton, an excellent physical frame of reference is provided for the generation of highly reproducible distortions. Not all forms of mechanical energy are detectable; static pressure, for example, is ineffective on the virtually incompressible cuticle (Schwartzkopf, 1974). The mechanical energy to which chordotonal organs are
sensitive involves static position, or changes in position, of cuticular parts. Ultimately, therefore, chordotonal organs detect displacement.

Invariably the displacement energy pathway from cuticle to scolopidial dendrite is indirect, because chordotonal organs are always located subepidermally. Unlike cuticular sensilla, such as hairs and campaniform organs, which can directly detect cuticular shear and torsion, the attachment and accessory cells provide chordotonal scolopidia with a viscoelastic bridge to the cuticle. Nevertheless, the range of cuticular displacement to which insect chordotonal organs respond includes seven orders of magnitude, from about 6x10^-10 m for tympanal organs (Michelsen and Larsen, 1985) to 1.3x10^-3 m for the locust FeCO (Field and Burrows, 1982; and unpublished observation). A variety of histological mechanisms has evolved in insects to adapt the sensitive scolopidium to such a great range of mechanical inputs. These allow, for example, impedance matching or rectification of high frequency oscillations in tympanal organs (Suga, 1960; Howse, 1964), tuning of Johnston’s organ through antennal resonance (Gewecke and Schlegel, 1970), filtering by rejection or dispersion of inappropriate aspects of a mechanical signal, leaving a physiologically adequate signal, as in the acceleration-sensitive SGO (Schnorbus, 1971; Shaw, 1994a), mechanical amplification of effective movement range in the notonectid tarsal chordotonal organ (Wiese and Schmidt, 1974), and viscoelastic reduction of movement by the ligament in the locust FeCO (Field, 1991; Shelton et al. 1992).

7.3 METHODS OF ANALYSIS

Mill and Price (1976) reviewed ways to describe and analyse proprioceptor systems. Other papers, including more recent reviews, may be found in Thorson and Biederman-Thorson (1974), Marmarelis and Marmarelis (1978), Bohnenberger (1981), Naka et al. (1985), Hofmann et al. (1985), Michelsen and Larsen (1985), Matheson (1992b) and Marmarelis (1993).

Two basic approaches have been used to study the physiological responses of chordotonal organs. The first consists of delivering a range of mechanical stimuli to the intact chordotonal organ through joint movement, sound or vibration, and describing the spike responses of sensory neurones as a function of various stimulus parameters. Some joint chordotonal organs have been detached from the cuticle and stimulated in an open loop configuration to achieve accurate control of the stimulus by preventing interference from feedback responses. Initially the extracellularly recorded responses of entire populations of sensory neurons were described, either as spike frequency versus joint position, velocity or acceleration (stimulus response curves) for joint chordotonal organs, or as intensity of sinusoid stimulus required to reach threshold (threshold sensitivity curves) in sound or vibration-sensitive chordotonal organs. Later, single unit intracellular studies revealed a rich variation in mechanosensitivity within chordotonal organs. The second
approach consists of delivering a characteristic forcing function as an input and using systems analysis mathematics to model the output by calculating transfer functions in the time or frequency domains. The forcing function can be (1) a step change, during which spike output is usually analysed against time with linear models, (2) a steady state sinusoidal stimulus during which phase and gain of spike output can be analysed in the frequency domain using Bode or Nyquist plots, (3) a random (white noise) stimulus (usually with a flat frequency spectrum and a Gaussian distribution of amplitudes) against which the spike output is compared using cross-correlation and spectral analysis in the frequency domain (Mill and Price, 1976; Kondoh et al. 1995).

7.4 RESPONSE PROPERTIES

Descriptions of chordotonal response properties have been embedded in reviews of mechanoreceptors or of acoustic receptors. Schwartzkopf (1974), Wright (1976) and Finlayson (1976) provided early reviews of joint chordotonal organ physiology of insects (useful comparisons with crustacean chordotonal organ physiology can be found in Mill (1976) and Wales (1976)). Bässler (1993) reviewed the current knowledge of the stick insect leg control system, including FeCO physiology. Tympanal organ and subgenual organ (SGO) physiology have been reviewed by Elsner and Popov (1978), Michelsen and Larsen (1985), Oldfield (1985a,b), Dambach (1989) and Boyan (1993). Recent texts dealing with acoustic and vibrational communication (Huber and Markl, 1983; Kalmring and Elsner, 1985; Huber et al. 1989; Bailey and Rentz, 1990) also include information on tympanal organ physiology, so these receptors will not be covered extensively in the present review.

7.4.1 Tonic versus phasic responses recorded in whole nerve

Early extracellular studies established the nearly universal presence of two types of neurons in arthropod chordotonal organs: tonic units that code for static joint position through steady prolonged discharge, and phasic units that discharge during joint movement, sound or vibration (Usherwood et al. 1968; Young, 1970; Schnorbus, 1971; Burns, 1974). Such studies dealt with large or easily-recorded chordotonal organs such as the FeCO or the SGO.

In joint chordotonal organs, plots of whole nerve tonic response versus static joint position give V-shaped or U-shaped curves with the minimum output centred around the normal resting joint position in the intact animal (Usherwood et al. 1968; Burns, 1974). Setting the joint to a new position results in a large transient discharge that adapts to a new resting level that is approximately linearly related to joint position. Whole nerve tonic spike frequency increases as joint position moves away from the normal rest position of 60°, and phasic spike frequency increases with increasing velocity (Usherwood et al. 1968; Burns, 1974). Responses of tonic units to steady state
displacement remain stable for up to 2 h. Phasic units adapt rapidly after step displacements, but fire steadily in response to maintained sinusoidal stimulation up to frequencies of 170 Hz for the locust FeCO (Usherwood et al. 1968).

It was assumed, correctly, that such whole nerve responses consisted of range fractionated responses of individual tonic and phasic units possessing a variety of response curves (see Section 7.4.2). Therefore the U-shaped curves from whole nerve recordings of spike frequency versus joint position do not indicate ambiguous afferent coding, since the range fractionation of individual units allows for unique line labeling of joint position. Furthermore, whole nerve recordings do not reflect accurately the contribution of small spikes coding midrange positions, and must be interpreted with caution (Hill, 1980; Matheson, 1992b).

The tarso-pretarsal chordotonal organ in the backswimmer *Notonecta* has three phaso-tonic neurons in the proximal scoloparium which respond to movement of the unguitractor plate, whereas five purely phasic neurons of the distal scoloparium respond to movement of either the anterior or posterior claws (Wiese, 1972).

The monocondylic joint of the coxa allows high mobility encompassing movements and positions in the three body planes. Evidently mesothoracic coxal afference is of greater importance than that from the same joint in other thoracic segments, since the complex chordotonal organ system (COS) is lacking in the pro- and metathoracic segments (Section 3.3.3). The mesothoracic coxal and thoracic chordotonal organs (ajCO, pjCO and the COS, Section 3.3.3) receive combinations of complex mechanical inputs resulting from interactions between forces acting along dispersed receptor strands (Fig. 6b). All of the chordotonal organs show movement sensitivity, but only the anterior chordotonal organ (ajCO) has position-sensitive units. The ajCO has attachments to at least six points on the coxa and thorax, and its tonic afferents code for position in any of the three planes with maximum discharge for sustained posterior rotation of the proximal ventral coxal rim. Coxal movement is coded by unidirectional phasic neurons; one group fires during coxal protraction (anterior movement) and another group fires during retraction (posterior movement) (Hustert, 1983).

The posterior joint chordotonal organ (pjCO) lacks tonic units. Its 5-7 sensory neurons are entirely phasic and respond to both directions of movement in the three planes of coxal displacement, or to inward bulging of the coxal membrane (Hustert, 1983).

The complex interlinked mesothoracic COS system (aCO, pCO, cCO, vCO) lacks tonic units, but has phasic units that code for a variety of coxal and thoracic movements. Thus the aCO, which attaches to the prothoracic spina, is stretched primarily during flight, hyperventilation, grooming and peering, and fires phasically during leg protraction in walking. The cCO strand links all four chordotonal organs to the coxa. During leg protraction (from posterior-medial
to anterior-lateral) the strand relaxes, and during retraction it is stretched. The aCO and vCO respond during both directions of movement, whereas cCO and pCO respond primarily during relaxation. The phase relationships between spike bursts and the stimulus depend markedly on the part of the natural movement range through which the cCO strand is moved (Hustert, 1978, 1982; Hustert et al. 1981).

The wing-base chordotonal organs in the cricket and locust are highly sensitive to vibration of the adjoining cuticle as well as to low frequency (2-5 kHz) sound (Möss, 1971; Pearson et al. 1989).

Tympanal and subgenual organs generally respond with V-shaped curves of spiking threshold versus sound (or vibration) stimulus frequency, which indicates specific tuning of the organs to a narrow frequency band at the threshold minimum (e.g. Schnorbus, 1971; Field et al. 1980). Intracellular studies established that such curves are made up of overlapping responses of individually tuned neurons with relatively narrow ranges, each covering part of the entire dynamic range of the organ (range fractionation).

In summary, recordings of whole nerve responses established that joint chordotonal organs code for position (tonic responses), movement (phasic responses), and direction of movement, whereas tympanal and subgenual chordotonal organs code for sound frequency or vibratory acceleration. However, whole nerve responses can only indicate the boundaries of stimulus parameters to which a chordotonal organ is responsive (e.g. thresholds of sensitivity to movement, or maximal velocity of movement); they do not indicate details about the sensitivity thresholds, specific adequate stimuli, range fractionation, nor stimulus-response curves of individual scolopidial neurons. Such information has mostly appeared in the past decade and is based upon intracellular recordings from single chordotonal axons or neuron somata.

7.4.2 Single unit responses


7.4.2.1 Joint chordotonal organs FeCO scolopidia are able to code complex attributes of the mechanics of the femoro-tibial joint. FeCO neurons from the stick insect and locust have been classified into no less than 22 different
Table 5. Response types of FeCO neurons from the stick insect and the locust. Current interpretation (Kondoh et al. 1995) suggests that these categories are artificially narrow and some should be more realistically described as in the right hand column. Tonically firing neurons that coded for position (i.e. static joint angle) are designated 'P'. Joint position at which maximal response was reached is indicated as '+' for flexed, '-' for extended and 'm' for mid-position. Neurons that coded for velocity of tibial movement are designated 'V', while those that coded for acceleration are designated 'A'.

Unidirectional movement sensitivity is designated as '+' for flexion and '-' for extension of the tibia, and bi-directional sensitivity is designated as '±'. 'AS' indicates acceleration units that fired at the onset of movements in any direction but not at termination. (low) and (high) refer to low (max.=27 Hz) and high (max.=58-117 Hz) frequency white noise stimulation at which the response types were obtained. Arrows indicate neurons whose classification altered when the stimulation frequency was increased. Boldface numbers indicate more than five recordings.

<table>
<thead>
<tr>
<th>Category</th>
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<th>Locust</th>
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<td>(2)</td>
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<tr>
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<td>Pm</td>
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<td>1</td>
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<td>P-</td>
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<td></td>
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<td>P-V-</td>
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<tr>
<td></td>
<td>AS</td>
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</tbody>
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Sources: (1) Hofmann et al. (1985); Hofmann and Koch (1985); (2) Büschges (1994); (3) Matheson (1990, 1992b); (4) Kondoh et al. (1995).
categories of mechano-sensitivity (Table 5). In the stick insect midleg (Büschges, 1994), hind leg (Hofmann et al. 1985; Hofmann and Koch, 1985) and the locust hind leg (Matheson, 1992b), the categories were thought to be stable. A major new insight is that some scolopidial units change response category as the frequency of sinusoidal stimulus movement changes (Kondoh et al. 1995) (see Section 7.4.2.2). Büschges (1994) focused on the ventral scoloparium of the stick insect only, and confirmed and extended earlier results on the whole FeCO.

The classification of neurons into the categories of Table 5 does not mean that the neurons themselves are capable of discriminating the various mechanical parameters of the response types. Certainly each scolopidium shows such discrimination, but it is an open question whether the viscoelasticity
of the attachment cells, the ultrastructure of the distal dendritic segment, the membrane conductance channels in the neuron, the compliance of the scolopale or a combination of these factors, confers the mechanical specificity of the scolopidial response. Furthermore, in view of the results of Kondoh et al. (1995), the response categories must be viewed as artificially convenient. In reality, some units operate in a frequency-dependent continuum encompassing more than one category. For convenience, the categories will be used in the following discussion, but with the above proviso in mind.

The following generalisations apply to the data in Table 5. The FeCO preferentially responds to flexed tibial positions and flexion movements with many more position- and velocity-sensitive units encoding these positions and movements than there are neurons encoding extension movements or position (Fig. 22a). Apparently this bias is not related to leg shape or function (middle vs hind leg). Often the position-sensitive units are small neurons with tiny axons and small spike amplitudes (Zill, 1985a; Hofmann et al. 1985; Matheson, 1990). Although some show no sensitivity to movement, others become velocity-sensitive with rapid displacement (Kondoh et al. 1995). Nearly all position- and velocity-sensitive units have unidirectional velocity sensitivity (three in the stick insect were bidirectional, but no such responses are reported for the locust). There is a great variety of phasic and phaso-tonic response types, ranging from “mostly position-sensitive” to “mostly velocity-sensitive” (Hofmann et al. 1985; Matheson, 1990).

Pure velocity-sensitive units are most abundant, which presumably reflects the importance of reporting velocity of joint movement to motor control circuits in the CNS (see Bässler, 1988, 1993 for discussion of velocity control during walking). Other velocity sensitive units often show spontaneous firing (Fig. 22b) and show a position effect (Fig. 23b) whereby a progressive shift in starting positions of a constant stimulus movement gives progressively weaker responses for equivalent velocities (Hofmann et al. 1985; Matheson, 1990, 1992b).

Acceleration units are primarily extension-sensitive or bi-directional. The high proportion of acceleration units in the locust hind leg FeCO may indicate the importance of monitoring extension during jumping. The large axons and rapid spike conduction of acceleration units means rapid information transfer to the CNS during a jump (Hofmann and Koch, 1985). Most are not spontaneously active, only fire one or a few high frequency spikes at the onset or end of a movement (Fig. 22c,d), and are sensitive to very small movements (Hofmann and Koch, 1985; Matheson, 1990). These properties also confer vibration sensitivity on these units. An ultrastructural and electrophysiological comparison of the acceleration-sensitive units in the FeCO to the scolopidia in the vibration-sensitive SGO, and the proximal scoloparium of the mid- and front leg FeCO could shed light on understanding the mechanism of acceleration sensitivity (Schnorbus, 1971; Hofmann and Koch, 1985; Field and Pflüger, 1989; Shaw, 1994b).
Fig. 23. Complex response properties of tonic and phasic chordotonal organ neurons. 

a. Range fractionation in position-sensitive units from the locust metathoracic FeCO. Within the range of femoro-tibial joint angles shown, FeCO length-change is nearly linear (tibial flexion stretches the organ). Although some units show linear responses over part of their firing range (but never all of it), others are non-linear. One shows a central peak of firing. Some have increased firing with progressive FeCO relaxation while others have increased firing with progressive FeCO stretch.

b. Velocity units of the locust metathoracic FeCO show a position effect in which the response range increases as velocity increases. This extension-sensitive unit responds at more flexed angles as velocity increases, suggesting that there is a gain change proportional to velocity.

c. Sound-sensitive units of the bushcricket crista acustica (tympanal organ, shown diagrammatically below) have sharp response peaks rather than monotonic response curves. The responses are tonotopically mapped onto the crista acustica as seen by the positions of dye-filled neurones which gave the responses shown by the arrows.

d. Hysteresis in position-sensitive neurones of the locust metathoracic FeCO. Note that hysteresis differs for each example. One unit fires more strongly upon relaxation of the FeCO, another fires more strongly upon stretch, and a third shows a combination of the two hysteresis types. a, b, d modified from Matheson (1992b), with permission; c, modified from Oldfield (1982), with permission.
The extensive range of sensitivity to different mechanical parameters of the femoro-tibial joint should not be generalised to other chordotonal organs. For example, the coxal joint chordotonal organs, pre-tarsal chordotonal organs, and sound- and vibration-sensitive chordotonal organs show fewer kinds of response sensitivity.

**Range fractionation** In the FeCOs of both locust and stick insect, all response types show range fractionation. The most prominent range fractionation occurs in position-sensitive units (Fig. 23a), which vary from narrow-range units that respond mostly at extremes of tibial angles, to mid-angle units that have restricted ranges, to broad-range units that are sensitive to the entire range of joint positions (Zill, 1985a; Hofmann *et al.* 1985; Matheson, 1992b). Afferents with the narrowest response ranges predominantly signal extremes of tibial movement. Generally, though, response ranges are fairly wide. For example, Matheson (1992b) states that half of the tonic afferents fired at all tibial angles, and three quarters of the phasic afferents fired at all tibial angles when stimulated with the fastest movements.

Range fractionation in velocity-sensitive units exists in (a) the range of leg angles over which they fire in response to movements of a given velocity, and (b) the range of velocities over which neurons fire at a given leg angle. Fig. 23b illustrates the responses of an extension-sensitive neuron in which spike output and range of sensitivity increase at angles of less than 120° as velocity of movement over a 20° arc increases. This suggests that the gain of the neuron's input-output transfer function increases with velocity, as found by Kondoh *et al.* (1995) for certain velocity-sensitive units (see Section 7.4.2.2).

A mechanical basis for range fractionation occurs in the locust hind leg FeCO for distributing tension onto scolopidia sequentially as the tibia moves through its range of joint angles. The viscoelastic ligament that joins the FeCO to the tibia consists of strands (presumably single or grouped attachment cells) of different lengths which are sequentially tightened during tibial flexion, until all are under full tension at maximum flexion (Field, 1991; Shelton *et al.* 1992). Intracellular recordings show that some units respond to loading of strand tension, while others respond to unloading of tension (Matheson, 1990).

**Thresholds** In conditioned learning experiments, the locust is able to discriminate femoro-tibial joint angle differences of as little as 5° (56 µm stretch of FeCO), which provides a highly conservative estimate of the threshold sensitivity of position units in the FeCO (Hoyle, 1980). The minimum displacement required to alter the firing rate by a single additional spike appears not to have been investigated for tonic units. The minimum threshold for single velocity-sensitive units is around 4.4 µm s⁻¹ in the locust, and 3-4 µm s⁻¹ in the stick insect, although in the locust many velocity units have thresholds up to 350 µm s⁻¹ (Hofmann *et al.* 1985; Matheson, 1990). The sensitivity of the stick insect FeCO, however, is much less than that required to code for the 0.013 µm s⁻¹ tibial movement observed during cataleptic behaviour, which is controlled by feedback from the FeCO, suggesting that
detection of such minute velocities probably involves comparison of many inputs, as in the summation model proposed by Hofmann et al. (1985). Acceleration unit thresholds in the FeCO vary from 2 to 44 cm s\(^{-2}\) (Hofmann et al. 1985). The smallest values fall within the range observed for the subgenual organ of the cockroach (0.04–4 cm s\(^{-2}\)), which contains exclusively acceleration-sensitive scolopidia (Schnorbus, 1971; Dambach, 1989).

**Hysteresis** Hysteresis is a property of position- and velocity-sensitive units in the FeCO (locust: Usherwood et al. 1968; Burns, 1974; Zill and Jepson-Innes, 1988; Matheson, 1990, 1992b; stick insect: Hofmann et al. 1985). Units showing hysteresis fire at a certain rate for a given joint position when that position is approached from one direction, but fire at a different rate when the position is approached from the opposite direction (Fig. 23d). Hysteresis can be quite marked or barely detectable, as seen in the following examples. When the tibia was *extended* to a joint angle of 40°, the firing frequency of one position-sensitive unit in the locust was approximately 4 Hz (measured 3s after movement ceased), but the same unit fired at 25 Hz when the tibia was *flexed* to 40°. Another position-sensitive unit showed only a 2 Hz difference in firing rate when subjected to the same test. In most units, the tonic firing rate at a given angle is higher if that angle is approached by a movement that phasically excites the unit (Matheson, 1992b).

Since viscoelastic materials exhibit hysteresis, the first obvious interpretation of the above results is that the FeCO ligament introduces a direction-dependent phase lag to movement which results in directionally-dependent position-sensitivity. Unfortunately this interpretation is complicated by the observation that some units respond more strongly at a given angle if that angle is approached by a movement that phasically reduces the firing rate of the unit (Matheson, 1992b). Studies that characterise the viscoelastic properties and firing rates of identified units are required to reach an understanding of the basis of hysteresis in chordotonal organs.

### 7.4.2.2 Gaussian white noise analysis

A rapid and accurate method of determining the dynamic properties of sensory systems in the frequency domain is to analyse the neuronal response to a band-limited Gaussian white noise stimulus. Cross-correlation of single neuron spike responses to the input function yields Wiener kernels, of which first-order kernels give a description of linear components and second-order kernels describe non-linear components of the response. Fourier transformation of the Wiener kernels provides gain and phase information of linear responses in the frequency domain (Marmarelis and Marmarelis, 1978; Naka et al. 1985).

Wiener kernel analysis was applied to locust hind leg FeCO neurons by Kondoh et al. (1995) to provide not only a new description and interpretation of response types (Table 5) but also models of tonic and phasic responses. Gain curves (Bode plots) of neuron response *versus* frequency of input show that tonic units have a constant gain (i.e. gain is frequency-independent) with a
cutoff frequency of about 80 Hz. They are thus non-differentiating with respect to time and therefore position-sensitive. Phasic units have differentiating first- and second-order kernels, in keeping with their time-derivative sensitivity to velocity and acceleration. Bode plots of phasic units give gain curves of 20 dB/decade for velocity-sensitive units and up to 30 dB/decade for acceleration-sensitive units, with peak response magnitudes at 80 Hz. The non-linear component, revealed by the second-order kernels for both tonic and phasic units, is primarily a rectification, or directional sensitivity. Other non-linear components, such as threshold effects, occur at extremes of the stimulus frequency range as discussed in Section 7.4.3.2.1.

A major new understanding gained from this method of analysis is that some neurons change their sensitivity to physical movement parameters in a frequency-dependent manner. Thus some units that are position-sensitive at low-frequencies become almost purely velocity-sensitive at higher frequencies, and some units that are velocity-sensitive at low-frequencies become acceleration-sensitive at higher frequencies. An important consideration from the viewpoint of transduction is that only some units showed frequency-dependent changes in sensitivity, while others retained their single modality sensitivity. Is this a reflection of mechanical properties unique to some of the scolopidia and not others? As in other sinusoidal analyses, Bode plots also provide information on the phase relationship of the response to input over a range of frequencies. This was not presented in the locust FeCO study (Kondoh et al. 1995), but would be of great interest insofar as it could indicate the nature of a possible viscoelastic component in series with the neurons.

The response dynamics of the FeCO neurons were modelled as pairs of filters. For the tonic units the cascade consisted of a dynamic low-pass filter followed by a static non-linear filter (rectifier). The low pass filters have constant gain, as expected of a position detector (Kondoh et al. 1995). Phasic units comprised a dynamic band-pass linear filter and a static non-linear filter. In contrast, Bässler (1993) described the velocity-sensitive FeCO units of the stick insect as high-pass linear filters, presumably with unknown non-linear components as well (Kondoh et al. 1995).

7.4.2.3 Further research Understanding is lacking for the following mechanisms underlying the responses summarised in Table 5. It is not clear why some position units have no response to movement whereas others show different degrees of movement sensitivity in addition to position sensitivity. Another problem lies in understanding why movement-sensitive units show different classes of sensitivity (pure V or A units versus VA units). A related problem is why some movement-sensitive units show an increase in response gain, or change in sensitivity from one modality to another, as frequency increases in a sinusoid stimulus (Kondoh et al. 1995). This may be related to the presence and nature of different mechanically-activated ionic conductance channels (see Section 6.1) in the neurons and/or to differences in viscoelastic
properties of the attachment cells. A further important problem is to understand the basis for uni- versus bi-directional movement sensitivity in scolopidia. Yet another problem is understanding the basis for hysteresis. All the above may be restating the same problem in different forms. It is obvious that the contribution of mechanical components in the energy pathway of mechanosensory detection must be elucidated.

It is also important to determine the contribution of ion channels to chordotonal neuron response properties. Some insight may be gained from unusual response types. For example, P-V+ units present an apparent paradox in which the position-sensitive response increases with FeCO relaxation, but the velocity-sensitive component of the response increases with stretch (Matheson, 1990, 1992b). Matheson (1992b) suggested that the two response components are somehow uncoupled and act independently.

7.4.2.4 Tympanal and subgenual organs Elsner and Popov (1978) extensively reviewed the physiological characteristics of insect tympanal neurons, and few additional new principles have come to light since then. The following summary will concentrate on these more recent developments.

Intracellular recordings from tympanal organs resolved the question of whether a sensory neuron responds at each cycle of the sine wave input to code frequency in a direct phase-locked manner, or instead is linked to some mechanical, perhaps resonant, property of the ear structure that causes it to fire preferentially at an optimum frequency. The latter is a version of the place principle seen in the vertebrate cochlea, where the physical constraints of the tapered structure cause different frequencies of sound to energise different regions of the basilar membrane and thereby allow, in effect, a Fourier analysis of frequency along the length of the cochlea (Michelsen and Larsen, 1985). The general answer to the above question is that tympanal neurons do not code sound frequency by phase-locking spikes onto the sine wave, but instead use spike firing rate to code for sound intensity (Elsner and Popov, 1978; Kalmring et al. 1978; Michelsen and Larsen, 1985). Vibration-sensitive neurons, however, phase-lock onto vibrational stimuli up to saturation of the neuron’s spiking rate at about 350 Hz. Above that stimulus frequency they must utilise the place principle for coding (Michelsen and Larsen, 1985; Dambach, 1989).

In contrast to the abundance of tonic position detectors in joint chordotonal organs, sound and vibration scolopidia are, by the nature of the stimuli, phasic receptors. The following generalisations may be drawn. The frequency sensitivity curves are V-shaped, indicating that scolopidia are more or less tuned to a characteristic frequency (CF) at the neuron’s lowest threshold (Fig. 23c). Nearly all organs show range fractionation of units in the overall frequency band to which the organ responds. Many neurons have high roll-off (20-40 dB) characteristics that allow for minimal overlap of individual tuning curves (Rheinländer, 1975; Hill, 1980; Oldfield, 1982; Kalmring et al. 1978,
others have broader response curves, but each still has a distinct CF (Kalmring et al. 1993). A new insight is that range fractionation is associated with tonotopic organisation of scolopidia in the crista acustica in tettigoniids, and probably in other ensiferans as well. As in the vertebrate cochlea, lower frequency units are found at the wider, proximal end of the crista acustica, and have larger somata, while progressively higher frequency units are found more distally as the crista acustica tapers and neurons become smaller (Oldfield, 1982; Lin et al. 1993; Kalmring et al. 1993). Another new discovery, which may turn out to be a general property of sound and vibration receptors, is that their neurons appear to fire in a synchronised, oscillatory fashion, particularly to click-like sounds of insect stridulatory calls. A similar phenomenon has been noted for vertebrate neurons in the visual cortex, and appears responsible for high sensitivity to critically-timed stimulus patterns in such systems (Elsner and Popov, 1978; Kalmring et al. 1990b; Rössler et al. 1990).

7.4.2.4.1 Receptor response types Previous work defined four groups of receptor types in Müllers organ in locusts: three are characterised by CFs at different low frequencies, but have different thresholds, and the fourth is sensitive to high frequencies (Michelsen, 1971, reviewed in Elsner and Popov, 1978; Michelsen and Larsen, 1985).

In tettigoniids, new work shows that the receptors of the crista acustica fall into five response types, based upon their CFs, and the shape of these frequency threshold curves: 1) CF 5-9 kHz, 2) CF 5-18 kHz, 3) CF 6-12 kHz, 4) CF 18-20 kHz, 5) CF 18-30 kHz. These functional types are common to five species examined, despite species differences in number of scolopidia in the cristae, the size of tympanal tracheae and the dominant frequency of the conspecific songs. Tuning to the characteristic songs of each species is accomplished by different distributions of receptor types (i.e. different “frequency weightings”) in each crista population of receptors (Kalmring et al. 1993). Thus there is still range fractionation in the distribution of response curves across the whole response frequency band (2-40 kHz), but not necessarily an equally-spaced distribution, as implied by Fig. 23c for example (Oldfield, 1982). The different ‘frequency weightings’ of crista acustica responses for the different species implies a common ancestry before divergence of the species (Kalmring et al. 1993; Lin et al. 1993).

Response types have not been demonstrated for individual receptors of subgenual organs (SGO), nor has there been a concerted effort to determine whether the scolopidia are physiologically different from those in the other parts of the complex tibial organ.

7.4.2.4.2 The basis for frequency tuning Controversial research on the basis for tuning of receptor types continues, and the solution is still not clear. In locusts, tuning is entirely dependent upon the resonant properties of different regions of the tympanum to which the four groups of scolopidia of Müller’s organ are attached. Intracellular recordings and laser vibrometry have confirmed that the
tympanum acts as a bandpass filter to allow sounds between 1 kHz and 40 kHz to stimulate the scolopidia. If the tympanum is bypassed and the organ is stimulated by controlled vibration, the sensory response range extends from 50 Hz to 100 kHz (Michelsen, 1971). Therefore, the four receptor response types appear to result from specific placement of neurones on the tympanum.

In contrast, Oldfield (1982, 1985a,b) presented evidence that in the crista acustica of tettigoniids, tuning to individual CFs is an inherent property of each sensory neuron, since it does not rely upon the resonant properties of tympanic membranes nor upon the structural continuity of the crista acustica. Recent conflicting results, based upon tuning curves, morphometric analyses and distribution of frequency response types of units in five species of tettigoniids, suggest that the width of the dorsal wall above the crista acustica, and of the tectorial membrane, are responsible for tuning through local resonances (Kalmring et al. 1990; 1993). The current interpretation is that the existence of apparent receptor response types (Section 7.4.2.4.1) is determined mainly by the distribution of receptors into regions of local resonances, rather than by intrinsic physiological properties of neurones. However, these conclusions resulted from ablation experiments, and still do not address the positive results shown by Oldfield. The question of whether electrical properties of cell membranes contributes to determination of response type is still open.

7.4.2.4.3 Synchronised responses A major new area of research in acoustic receptor physiology concerns synchronised and oscillatory responses. In bush crickets (Tettigoniidae) but not in field crickets (Gryllidae) the transient sound pulses resulting from tooth impact during the song causes synchronised firing of the receptor ensemble of the crista acustica (Rössler et al. 1990; Nebeling et al. 1993; Rössler and Schul, 1993). Although synchronisation of firing in the tympanic nerve was known in locusts (Popov and Svetlogorskaya, 1971), its significance in coding transient aspects of conspecific sound was not investigated in detail. In tettigoniids, the pulse pattern within syllables of the song appears to be of significance in the receptor coding and central processing of afferent input. Individual neurons are highly sensitive to rapidly damped clicks, and respond with constant latency and stereotyped firing pattern. In Decticus verrucivorus, for example, single neurons fire at about 350 Hz to clicks repeated either at 3.2 ms intervals (1:1 firing) or to more closely spaced clicks at the saturation firing frequency of 350 Hz. The neuronal time constant of about 2.8 ms and refractory period are sufficiently short to allow stable driving by the 3.2 ms click stimulus. The stable response must have clicks presented at carrier frequencies to which the neurons are tuned, as occurs in the natural song; and, for different species, there are different time constants and optimal firing rates (Kalmring et al. 1990b). The cause of such highly sensitive stable responses to transient stimuli is thought to be related to resonances in the system, including complex resonant modes of the tympanum (Rössler et al. 1990).

Synchronised firing of afferents is observed in the tympanal nerve and in the
auditory neuropil of the prothoracic ganglion (Rössler et al. 1990; Nebeling et al. 1993; Rössler and Schul, 1993). As the transient sound pulse pattern of artificial songs approaches the pattern of natural songs, synchronisation and stability of the mass response increase. Furthermore, crista acustica neuron populations show species-specific filter properties to preferentially pass the conspecific song (Rössler and Schul, 1993).

Synchronised firing of subgenual organ afferents is implied by the field potential responses to vibrations recorded from the metathoracic leg nerve of the cockroach by Shaw (1994a). The first 10-15 ms of the response signal (vibration evoked potential, VEP) consist of a highly stable oscillation which later becomes mixed by non-synchronous biphasic spiking in the nerve. Intracellularly recorded responses to a 2.15 kHz stimulus show a highly stable firing of the first two or three spikes at 2.5-3.0 ms intervals, which then degrades into erratic firing of a few more spikes. The recordings came from sheath cells that are electrically coupled SGO neurones, so they probably represent the firing pattern of the neurons (Shaw, 1994b).

7.4.3 Stimulus intensity-response curves

Ideally the simplest spike coding of mechanical stimulus intensity would be a linear relationship between firing rate and displacement for tonic (steady state) chordotonal neurons, and a linear relationship between firing rate and velocity, acceleration or sound amplitude for phasic (dynamic state) chordotonal neurons (Mill and Price, 1976). However a major limitation with linear responses is that the neuron gives a small output to a change anywhere in its response range, since the output is equal for equal stimulus intensity steps in any part of the range. The maximal firing rate of the neuron (several hundred Hz) would limit the effective range of coding, and it would be difficult to encode even several of the seven orders of magnitude of known stimulus range for chordotonal organs. This problem could be overcome in chordotonal organs by two mechanisms: a logarithmic response characteristic, in which the increase in energy to give a constant increase in response at low stimulus levels can be several orders of magnitude larger at high stimulus levels, or by range fractionation in which individual scolopidia encode only part of the stimulus range, as discussed previously. Unfortunately, variations and exceptions to a generalised logarithmic intensity-response curve occur in chordotonal organs, underlining a complexity that requires extensive further research. No systematic review of this variation has appeared.

7.4.3.1 Tonic units  Tonic mechanoreceptors are characterised by a steady state output in the absence of a forced input, and no response to movement. If compared with the classical slowly-adapting position detector, the crustacean MRO, or the spider slit sense organ (Bohnenberger, 1981), chordotonal
Fig. 24. Stimulus intensity-response curves for phasic units from a variety of chordotonal organs, showing non-linear properties such as threshold, dynamic response range and saturation. All responses are plotted on semi-logarithmic axes except those of a, which are plotted on linear axes. a. Responses of three position-sensitive units from the stick insect mesothoracic FeCO showing linearity in only a small part of the response range. b. Three velocity-sensitive units from the locust metathoracic FeCO. c. Two velocity-sensitive units from the stick insect mesothoracic FeCO. Note saturation. d. Velocity-sensitive unit from the abdominal chordotonal organ of a stick insect. e. Crustacean propus-dactylus velocity-sensitive units, for comparison to insect response curves. Threshold and saturation shown. f. Sound-sensitive units from the bushcricket tympanal organ show clear threshold and saturation effects at a single stimulus frequency. g. Cockroach subgenual organ acceleration-sensitive unit with an abrupt threshold and a steep response curve. h. Three acceleration-sensitive units from locust metathoracic leg show saturation. Data taken from: a, c Büschges (1994); b, Matheson (1992); d, Orchard (1975); e, Mill and Lowe (1972); f, Oldfield (1982); g, Shaw (1994a); h, Kuhne (1982).
organ neurones should have a simple linear change of spike rate versus change in length over at least part of the stimulus range (Mill and Price, 1976). However the curves in Fig. 23a,d and Fig. 24a show that, at best, some units have only a partially linear response. Others show a wide variety of responses, which do not conform to linear, logarithmic or exponential relationships characteristic of other sensory systems. Furthermore, response curves of position-sensitive units are not necessarily monotonic. Some have a peak response in the middle of their range (e.g. Fig. 23a), as seen also in the phasic sound-sensitive units of tympanal organs (Fig. 23c). Many joint chordotonal organ tonic units fire more strongly towards one extreme of the position range of the joint (Hoffman et al. 1985; Matheson, 1992b; Büschges, 1994).

Presumably a major component of the response characteristics of position receptors should be related to the viscoelastic characteristics of the attachment cells and other mechanical suspensions between the scolopidia and the exoskeleton. In the simplest case, where viscosity has been overcome by the elastic component after sufficient lapse of time following experimental setting at different joint positions, a linear relationship (Hooke’s Law) might be expected between developed force and firing rate. However, it is unlikely that a similar relationship exists between displacement and firing rate, since non-linearities are found at many points along the transduction pathway in sensory receptors (Mellon, 1968; Thorson and Beiderman-Thorson, 1974; Mann and Chapman, 1975; Mill and Price, 1976). This area of understanding in chordotonal organs requires investigation.

7.4.3.2 Phasic units From a systems analysis viewpoint, phasic receptors are not linear, since they do not have an output for all initial-state input conditions (Mill and Price, 1976). In some cases part of the response characteristic of a phasic unit may be linear, but most often the response curves are highly non-linear and only appear to approximate a logarithmic or power function in the middle of the stimulus range, with non-linear threshold and saturation effects at extremes of the range.

7.4.3.2.1 Logarithmic responses On semi-log axes, a logarithmic response gives a linear relationship between firing rate and the logarithm of stimulus intensity. When published responses from a variety of chordotonal organs are re-plotted in this way (Fig. 24b-h), it is immediately apparent that some response curves are not logarithmic, and others are only partly logarithmic. The curves are characterised by three features (not seen in all cases): (1) an initial threshold at low stimulus intensities, (2) a linear dynamic region encompassing the neuron’s maximum sensitivity, and (3) saturation where the response levels off at high stimulus intensities.

The threshold is not always seen as a non-linear tail; responses with and without the low intensity tail occur in neurons of the tettigoniid crista acustica (compare Fig. 24f,g with Fig. 24h). The left-pointing tail is not a unique aspect of any specific receptor modality, as it occurs in joint velocity units, SGO
acceleration units and tympanal sound units (Fig. 24). Models of such threshold phenomena are discussed in Section 6.

The *dynamic region* is highly variable in several aspects, but in general it represents the approximately log-linear region referred to by many authors (e.g. Elsner and Popov, 1978; Kalmring et al. 1990b). The *dynamic range* can extend beyond two decades (Fig. 24b-d) or can be highly compressed to less than a factor of 10 from threshold to saturation, as in Fig. 24g,h. The slope of the dynamic region gives the *gain* of the receptor. Some auditory and subgenual organ units have very high gain characteristics but saturate quickly (Kalmring et al. 1990b; Shaw, 1994b), while many velocity units in joint chordotonal organs have lower gain and a broader dynamic range (Matheson, 1992b). The significance of the dynamic range and gain of such response curves has not been addressed, but presumably relates to the range of environmental stimulus intensities encountered by the insect.

*Saturation* is characteristic of most types of phasic units in all chordotonal organs studied. Since individual response curves of neurons in most chordotonal organs are distributed over the entire range of sensitivity, saturation effects do not detract from the absolute sensitivity of the organs (Elsner and Popov, 1978). Often the apparent lack of saturation in a neuron’s response simply indicates that the neuron was not tested at higher stimulus intensities. The physiological basis for upper limits to the dynamic range of a chordotonal neuron’s response is not understood. At the extreme, some acceleration-sensitive FeCO neurons (Hofmann and Koch, 1985), crista acustica neurons (Kalmring et al. 1990b; Rössler and Schul, 1993), and probably the FeCO velocity units in Fig. 24c, fire at a maximum rate limited by their refractory period. Other neurons (e.g. tympanal organ units in Fig. 24f,h) saturate at very low firing frequencies. In these cases there must be mechanical constraints, as discussed for neurons of the locust Müllers organ (Michelsen, 1971).

### 7.4.3.2.2 Power functions

Both linear and nonlinear power-law dynamics have been applied to sensory systems by several authors. In non-linear behaviour, the characteristic exponent *k* applies to the stimulus intensity (*I^k*) over several orders of magnitude. In linear power-law dynamics, the non-integer exponent applies to time (*t^k*) or frequency (*f^k*). Thorsen and Biedermann-Thorsen (1974) and Bohnenberger (1981) have reviewed the basis for such analyses.

### 7.4.3.2.3 The sigmoid form of response curve has been modelled by the Hill equation for several receptor systems (turtle retinal cones: Baylor et al. 1974; insect hair sensillum: Thurm, 1965; cockroach SGO: Shaw, 1994b). For the cockroach subgenual organ, a good fit to initial response-intensity data (Fig. 24g) is provided by:

\[
\frac{V}{V_{\text{max}}} = \frac{I^k}{(I^k + b^k)}
\]

where *V* is the response amplitude and *V_{max}* is its asymptote, *I* is stimulus intensity, *b* is a constant defining intensity at half-maximal response and *k* is a measure of the slope of the dynamic phase (Shaw, 1994b). For the SGO, *k*
ranges in value from 2.2 to 3.4, the high value of which reflects the narrow dynamic range of the system. Most other authors have not modelled the intensity-response curves of chordotonal organs, nor tested for goodness of fit of logarithmic or other relationships to the dynamic phase of curves. Matheson (1992b) found that, rather than fitting the data for velocity units to a log-linear plot (as seen in Fig. 24b) higher linear regression coefficients were obtained with a log-log relationship:

\[(\text{firing frequency}) = 10^a \cdot (\text{velocity})^b\]  

(2)

where \(a\) is the slope of the regression line and \(b\) is the Y intercept. Values for \(a\) and \(b\) ranged from 0.25 to 1.55, and -2.52 to 1.44, respectively.

Another form (seldom considered in analyses of chordotonal receptors) of coding for intensity is the fractional power-law function. This function gives a straight line when spike rate is plotted against stimulus intensity on log-log axes (Thorsen and Biedermann-Thorsen, 1974). It can be demonstrated by plotting either the decay in firing rate to a step input function (time domain), or the dynamic firing of a neuron in response to different frequencies of sinusoid movement (frequency domain). To a step function, the decay in firing rate changes as \(t^k\) (where \(t\) is time and \(k\) is a constant between 0 and 1), and in the frequency domain the firing rate involves the \(k^{th}\) power of frequency and corresponding linear differential equations which are of fractional order. Analyses using fractional power-law dynamics have been carried out for several sensory receptors (not chordotonal organs) (Thorsen and Biedermann-Thorsen, 1974; Bohnenberger, 1981). An interesting aspect of power-law dynamics is that they describe linear processes: if the input is doubled, the response is doubled and the shape of the response waveform to a transient is unchanged. The analysis is therefore useful for experiments incorporating sufficiently small stimuli in the nearly linear ranges of receptors (Thorsen and Biedermann-Thorsen, 1974). The attraction for analysis of chordotonal organs is that such dynamics are characteristic of polymer viscoelasticity and could help explain the properties of the mechanical components of chordotonal organs. Although chordotonal organs have never been tested directly for fractional power-law dynamics, the re-analysed published records and graphs from two studies confirmed a power-law function for both (Bohnenberger 1981, Table 1). For the cockroach SGO the exponent, \(k\) (=0.6), was calculated from threshold curves and phase responses; for the tarso-pretarsal chordotonal organ of the backswimmer Notonecta, \(k\) (=0.5) was calculated from a step response (Bohnenberger 1981). Future experimentation should consider power-law analysis as a way to not only characterise the mechanical components underlying single unit response properties, but also to isolate those components from bioelectrical membrane components which contribute to the unit’s response. Such analysis could enhance the understanding of chordotonal organs with distributed-tension ligament systems (e.g. the hind leg)
7.4.4 Conclusions and future research

Physiological responses of chordotonal neurons can be analysed in terms of threshold sensitivity to mechanical stimuli or in terms of suprathreshold spike discharge rate to stimulus intensity. Threshold response curves have been used to characterise the phasic units of sound- and vibration-sensitive chordotonal organs, while spike rate has been used to characterise response-intensity curves of units in joint chordotonal organs. Although logarithmic functions may describe some aspects of such curves over broad stimulus ranges, chordotonal neurons do not follow a simple single dynamic response function. Instead, they appear to have a variety of response curves including linear, logarithmic and power function dynamics. Little has been done to analyse and model the response properties of chordotonal organs with a view to understanding the basis of spike encoding of mechanical stimulus parameters. The systems analysis approach has made a beginning, but much remains to be done. For chordotonal organs it is critical to investigate the properties of viscoelastic suspension and attachment structures which lie in series with scolopidia, and which undoubtedly influence receptor output. Also it is necessary to investigate the stimulus encoding mechanisms of the transducer components of chordotonal organs. Although a small start has been made (Section 6) much information is lacking compared to our understanding of other arthropod sensory systems.

8. Central projections

8.1 Overview

An important step in understanding the processing of information from any sense organ is to determine the anatomy of the receptor neurons’ projections within the CNS. This information can be assessed in the light of what is known of the structure and function of different regions of neuropil, and may suggest the types of postsynaptic neurons that could be directly influenced by the sensory afferents (i.e. those whose branches overlap). More reliably, the anatomical information may be used to rule out direct connections to putative postsynaptic neurons that do not branch in the areas containing the afferent projections. Investigations of afferent projections within an area of neuropil can indicate whether they are spatially ordered to form a representational map. Ultrastructural investigations can demonstrate whether afferent neurons make output synapses in only restricted regions of their overall branching area, the nature of these synapses (chemical or electrical), and whether the neurons
receive presynaptic input synapses. Coupled with immunohistochemical techniques, these approaches may also reveal information about the types of neurotransmitters present in the pre- and postsynaptic neurites.

The central projection patterns of a range of locust chordotonal organs have been reviewed by Pflüger et al. (1988). Grosch et al. (1985) have summarised the projections of vibrational receptors from the legs of the locust, Michelsen and Larsen (1985) have summarised and compared the central branching of auditory afferents in a range of Orthoptera, and Boyan (1993) has described the homologies between tympanal chordotonal organs of insects in several orders.

Merritt et al. (1993) and Merritt and Whittington (1995) describe the pattern of central projections of chordotonal organs in normal Drosophila melanogaster embryos, and in the former paper describe those following mutations of the cut gene (which transform external sensory [es] neurons into chordotonal sensory [cs] neurons; see Section 11.1). The development of femoral chordotonal organ projections (Lakes-Harlan and Pollack, 1993) is discussed in Section 10.5.3.

8.2 NEUROPIlar AREAS CONTAINING CHORDOTONAL ORGAN PROJECTIONS

Auditory and vibrational receptors of locusts and bushcrickets project primarily to a medial region of the ganglion traditionally called the anterior ring tract (aRT; Tyrer and Gregory, 1982). Pflüger et al. (1988) proposed that the area be renamed the medial ventral association centre (mVAC) to conform with well established terminology, and to indicate that the area is a neuropil in the true sense and not a tract. Almost simultaneously, Römer et al. (1988) proposed that this same area be termed the anterior intermediate sensory neuropil (aISN). For consistency with the bulk of other literature describing the anatomy of the CNS, the term mVAC should be used.

Briefly, mVAC contains branches of thoracic, abdominal and coxal chordotonal organs, including the auditory receptors (see Section 8.5) and the wing chordotonal organ. Some receptors from the mesothoracic femoral CO and from the subgenual organ also project to this region. There is no evidence for projections to this region from the metathoracic FeCO (but see Section 8.5.1). The lateral association centres (aLAC, pLAC) are the main neuropil regions to contain branches of the femoral CO and apCO. Chordotonal organ projections are not found in vVAC, LVAC or aVAC.

8.3 COMPARISONS BETWEEN SPECIES

The central projections of chordotonal organs in insects other than crickets and bushcrickets have not previously been reviewed in detail, but two reviews (Michelsen and Larsen, 1985; Boyan, 1993) have begun this task by summarising the projections of tympanal chordotonal receptors in Acrididae,
Tettigoniidae, Mantidae, Gryllidae, Cicadidae and Noctuidae. Boyan provides a further comparison with the atympanate dipteran *Drosophila*. There is clear homology between body chordotonal organs in *Drosophila* and those (including the abdominal tympanal receptors) of the grasshopper (Meier *et al.* 1991). This includes the areas of central branching. Further homology is proposed for similar neurons in the moth, mantid and cockroach (see Boyan, 1993 and Section 12).

8.4 CENTRAL ORGANISATION OF AUDITORY AND VIBRATORY CHORDOTONAL AFFECTENTS

The arrangement of auditory and vibrational afferents within mVAC of the locust, cricket and bushcricket has been summarised by Michelsen and Larsen (1985), but here we add more recently elucidated details of the tonotopic organisation within mVAC.

The tympani of crickets and bushcrickets are located on the tibia of the forelegs, whereas those of locust are located on the first abdominal segment. The projections of the afferent neurons are, therefore, correspondingly different. Nevertheless, there are many similarities, as discussed below.

8.4.1 Bushcricket and cricket

In the bushcricket, there is tonotopic mapping of the auditory receptors within mVAC (Römer, 1983; Römer *et al.* 1988; Oldfield, 1982, 1983). There is a clear difference in the projections of vibratory afferents from the complex tibial organ (subgenual and intermediate organs) and auditory afferents from the crista acustica (Römer, 1985; Kalmring *et al.* 1990a).

Recent work has documented that the tonotopic mapping of the auditory receptors within mVAC is more subtle than previously recognised (Römer *et al.* 1988). Tympanal afferents sensitive to the lowest frequencies (near 4kHz) project to the anterior-most region of the neuropil (Fig. 25ai). Afferents that respond best to frequencies of 8kHz and 20kHz project progressively more posteriorly along the ventral limit of the neuropil (Fig. 25aii,aiii,bi).
Fig. 25. a. In the bushcricket, auditory receptors with different ‘best frequencies’ project in an ordered way within the prothoracic mVAC, seen here in parasaggital sections and, in bi, a horizontal section. Vibration (and sound + vibration) sensitive chordotonal organ afferents also branch within mVAC, but they project more anteriorly or posteriorly, and have additional branches outside this area of neuropil (bii, biii). c. Auditory receptors from the abdominal tympanal organ (Müller’s organ) of locusts project differently within mVAC of the three thoracic ganglia (T1-T3). The four types have their peripheral somata in different places within the auditory organ, and their response properties differ. Their central projections are correspondingly segregated, thus forming a somatotopic representation within the CNS. Types 1, 2 and 3 are low frequency receptors: Type 1 are very insensitive; Type 2 are the most sensitive; and Type 3 have a somewhat higher characteristic frequency (6 kHz as opposed to 2-4kHz). The 8-10 Type 4 neurons are most sensitive to higher frequencies, above 10kHz. The left column represents sections taken horizontally through mVAC. Parasaggital sections at the levels indicated by a and b are represented in the two columns to the right. a, modified from Römer et al. (1988); b, modified from Römer (1985); c, modified from Halex et al. (1988).
Receptors most sensitive to frequencies of 30kHz have branches restricted to the most posterior, and slightly more dorsal, neuropil (Fig. 25aiiv). Finally, afferents sensitive to the highest frequencies project to the most dorsal region of the neuropil, anterior to the 30kHz receptors (Fig. 25av). The tonotopic map thus spans both anterior-posterior and dorsal-ventral axes, and not just the former as previously supposed. The map is best thought of as representing frequency in a counterclockwise pattern, starting with low frequencies in the anterior positions.

In addition, receptors tuned to frequencies near 20kHz have the largest arborisations (Fig. 25aiii). The song of *Tettigonia viridissima* contains a peak at this frequency, and it is here that directional sensitivity is greatest (see Römer, 1983). Ahi *et al.* (1993) studied *Psorodonotus illyricus, Decticus albrifrons* and *D. verrucivorus*, which have stridulatory songs with characteristically different basic frequencies. They showed that, in contrast to the afferents of *T. viridissima*, there are no major differences in the branching patterns or branch volume of equivalent auditory receptor fibres (i.e. with the same frequency tuning) in the three species. Nevertheless, because there are more low frequency receptors in the tympanal receptor of *P. illyricus* (which has the lowest frequency song), there is in this species a correspondingly greater representation of low frequencies in mVAC. It is also clear that there is considerable overlap of the branches of the receptors. For example, three receptors from the total population of 46 have a combined branching area that spans most of the neuropil (Ahi *et al.* 1993). Ebendt *et al.* (1994) point out that even pure auditory receptors in the bushcricket *Psorodonotus illyricus* have some branches outside mVAC. The implication from this is that these receptors may have more widespread synaptic connections than is generally accepted.

Vibration (or mixed vibration/auditory) receptors of the subgenual organ and intermediate organ have different central projections to those of the tympanal organ. Römer (1985) showed that these receptors (with best frequencies of 300 and 800 Hz in his example) branch along the length of their axons (i.e. before reaching mVAC), and that they then have a tuft of denser branches in the “posterior lobe of aRT” (Fig. 25bii), a region that does not receive auditory inputs. The fibre tuned to 800 Hz, but not that tuned to 300Hz, had a further region of branching along the anterior lateral margin of mVAC. This latter neuron was a bimodal auditory/vibratory afferent, since it responded also to low frequency airborne sound up to 5kHz. This anterior projection thus corresponds well with the anterior projections of pure auditory receptors that respond to equally low frequency sounds (Fig. 25bi). There is as yet no evidence for any ordering of the branches within the “posterior lobe”. Ahi *et al.* (1993) state that bimodal auditory/vibrational receptors (i.e. afferents from the intermediate organ) branch in the anterior region of mVAC but have an additional branch that abuts mVAC “caudo-laterally”. It is not explicitly stated if this is equivalent to Römer’s “posterior lobe”, but this appears to be the case. Lakes-Harlan *et al.* (1991) have investigated the auditory system of an
atympanate bushcricket, *Phasmodes ranatrichorpes*. Cobalt backfills of the leg nerve reveal projections in mVAC, along with projections in other neuropils. It appears likely that afferents from the crista acustica project to regions similar to those of their counterparts in tympanate species. Interestingly, in *P. ranatrichorpes*, the prothoracic mVAC is not enlarged as it is in tympanate bushcrickets, presumably reflecting the reduced requirement for central processing of the incoming information. In fact, it more closely resembles the homologous neuropils in the meso- and metathoracic ganglia of other species (i.e. of segments whose legs that do not possess auditory organs).

8.4.2 Locust

8.4.2.1 Auditory receptors The abdominal auditory apparatus of the locust *Locusta migratoria* consists of 60-80 receptors arranged into four groups (Müller’s organ) with different frequency responses and thresholds (Michelsen, 1971; Römer, 1976). Three of the groups respond best to low frequencies (near 4 kHz) whereas the other responds best to frequencies of 10-20 kHz. Cobalt fills of the entire nerve branches reveal that the afferent neurons enter the first abdominal neuromere of the metathoracic ganglion where they branch before projecting to the three thoracic ganglia. Here, the afferents project to regions of the neuropil that appear homologous to those containing the projections of tibial tympanal afferents in Tettigoniidae (i.e. mVAC).

Römer (1985) states that his fluorescent dye fills of individual receptors show that each neuron sends branches into all four neuropils, irrespective of whether it is a high or low frequency afferent. Halex *et al.* (1988), however, show that this is not the case. Their cobalt stains show that most of the low frequency afferents project only as far as the mesothoracic neuromere (some go only as far as the metathoracic neuromere), whereas high frequency afferents ascend to the prothoracic ganglion (Fig. 25c). The predominance of low frequency afferents in Müller’s organ may be compensated by the denser and more extensive branching of high frequency afferents within the four auditory neuropils. In addition, because some of the low frequency afferents, but generally not high frequency afferents, terminate in the metathoracic and mesothoracic ganglia, the representation of high frequencies becomes progressively more prominent in the anterior ganglia. Presumably this results in a similarly changing bias in the frequency characteristic of the auditory information arriving in each location.

The area of branching in the metathoracic mVAC is the most prominent and, here at least, there are differences in the projection areas of high and low frequency afferents. Römer (1985) shows that high frequency afferents project most posteriorly, in a sickle-shaped pattern when seen in sagittal section. Low frequency afferents from each of the other three groups project to distinct but overlapping regions further anterior. Within these regions, there
appears to be mapping according to threshold sensitivity to low frequencies, with the least sensitive afferents projecting furthest anterior. Halex et al. (1988) and Bickmeyer et al. (1992) provide additional information about the shape of the projections seen in both horizontal and vertical (sagittal) sections. From their work it is apparent that the most sensitive low frequency afferents project more medially than do the less sensitive afferents, rather than more posteriorly (Fig. 25c). This mapping is somatotopic, because it primarily reflects the peripheral location of the receptor. What has not been shown so far is tonotopic mapping of afferents from within a given receptor group.

The most antero-ventral (i.e. near the anterior ventral commissure, AVC) and dorsal (except posterior dorsal) regions of mVAC in the locust do not appear to contain branches of tympanal afferents (Römer, 1985). The anterior areas contain branches of pleural and coxal chordotonal organs.

Following transection of the tympanal nerve, auditory afferents can re-establish connections with appropriate interneurons (Lakes and Kalmring, 1990). When the regenerating fibres enter the metathoracic ganglion through the normal nerve root (n6) they project to the usual regions of the neuropil (e.g. mVAC, as outlined in Section 8.4 above). When the entry point is more anterior (through the leg nerve (n5) for example), the regenerating fibres generally do not grow posteriorly to innervate mVAC in the abdominal neuromere. The anterior projections, however, are more normal, innervating metathoracic mVAC and projecting to the correct locations in the anterior ganglia. Within approximately 2 months of nerve transection, most animals have a well innervated mVAC that is almost indistinguishable from that in intact animals. Even so, the projections of the four receptor groups are less clearly defined in the regenerated animals than in intact specimens, and individual afferent projections appear abnormal. Following unilateral nerve transection, intact tympanal receptors on the contralateral side of the animal often begin to produce projections into mVAC on the side of transection. These projections, which extend approximately 150 µm across the midline, do not subsequently retract when the regenerating fibres begin to re-innervate mVAC. The regenerating fibres are able to connect to appropriate ascending interneurons (TN-3, AN-1), but the auditory responses of these interneurons is somewhat different to normal (Lakes and Kalmring, 1990).

Interestingly, the wing chordotonal organ of Locusta migratoria, at least, also responds to low frequency (3kHz) sound (Pearson et al. 1989). Tyrer and Altman (1974) illustrate the combined projections of the wing stretch receptor and wing chordotonal organ, whereas Tyrer (1983) illustrates in diagrammatic transverse section the combined projections of the wing chordotonal organ and other wing sensory structures, including campaniform sensilla and hairs. Pearson et al. (1989) illustrate the projections of individual hindwing chordotonal organ afferents. These neurons project initially into the metathoracic ganglion before bifurcating to send a branch into the ipsilateral anterior connective. Within the metathoracic ganglion the neurons either
project directly into mVAC, or turn posteriorly before sending secondary branches medially into this region. Some neurons that follow this latter course also project posteriorly into the abdominal neuromeres, but their branches here are sparse. The projections in mVAC are sometimes bilateral. The morphology of individual wing chordotonal organ afferents within the mesothoracic ganglion is not shown, but the whole nerve backfill shows that they must be restricted to the medial part of the ganglion, near MVT and VMT. Some branches may cross the midline here also. There is no evidence that any axons project further anterior than the mesothoracic ganglion. The relationship between branching morphology and response type has not been examined.

Orona and Agee (1987) illustrate the projections of wing afferents in the moth *Heliothis zea*, but the complexity of the stained projections prevents a clear determination of which belong to the chordotonal organ.

8.4.2.2 Vibration receptors Vibration receptors in the legs of locusts have been classified by Kühne (1982) and their central projections summarised by Grosch *et al.* (1985). They probably include campaniform sensilla (Type I, Kühne, 1982), unidentified “leg joint receptors” (Type II) and the subgenual organ (Types III, IV). Notably, none of the vibration receptors illustrated by Grosch *et al.* (1985) project to the “auditory neuropil” (i.e. mVAC), so the vibratory information carried by these receptors must reach the known bimodal vibratory/auditory interneurons through an indirect pathway. Mücke and Lakes-Harlan (1995) show the projections of the mesothoracic subgenual organ (Fig. 26d), and suggest on the basis of intracellular stains that there are two basic patterns of branching: in one, the axon bifurcates at the lateral boundary of the neuropil whereas in the other, the axon bifurcates further medial. These differences appear slight, and receptors of the two proposed morphological types have indistinguishable responses to vibration. Clearly further work is required to substantiate these suggested differences.

Interestingly, Field and Pflüger (1989) suggest on the basis of physiological observations that the proximal scoloparium of the femoral chordotonal organ in *Locusta migratoria* is a vibration receptor, probably representing the Type II neurons of Kühne (1982). Axons from these neurons do project to mVAC, where they terminate in a dense plexus reminiscent of auditory projections (Fig. 26eii). It would now be appropriate to investigate whether FeCO neurons from this proximal scoloparium make direct connections with auditory/vibratory interneurons. The large number of receptors in the proximal scoloparium, and their potential to make direct connections with auditory/vibratory interneurons suggest that they may be a major component of the vibratory sense of the locust. The physiology and projections of neurons in the comparable scoloparium of the hind leg have not been described.

Grosch *et al.* (1985) have shown that the response characteristics and central projections of chordotonal and subgenua organ vibration receptors do not
Fig. 26. a. The afferents of a recently described auditory receptor located on the prosternum of the dipteran *Ormia ochracea* project to discrete medial regions of the first three neuromeres of the thoracic ganglion. b. Afferents from the prothoracic aCO of the locust *Locusta migratoria* (located in the anterior ventral part of the prothorax) also project to discrete regions (mVAC) in the first two thoracic ganglia, and to a larger region of the metathoracic ganglion. c. Afferents from the mesothoracic tibio-tarsal chordotonal organ of the locust *Schistocerca gregaria* project more laterally than do afferents from the tibial subgenual organ (d) or the distal scoloparium of the femoro-tibial chordotonal organ (ei). Afferents from the proximal (vibration sensitive) scoloparium of the femoro-tibial chordotonal organ project to the medial ventral association centre (mVAC) (eii) and not the lateral regions that contain the proprioceptive afferents from the same organ (ei). Auditory chordotonal organ afferents from the crista acustica of the bush cricket *Psorodonotus illyricus* also project to this region (f), as do those of the aCO (b). a, Modified from Robert *et al.* (1994), with permission; b, modified from Pflüger *et al.* (1988), with permission; c, modified from Laurent (1987a), with permission; d, modified from Mücke and Lakes-Harlan (1995), with permission; e, modified from Field and Pflüger (1989), with permission; f, modified from Ebendt *et al.* (1994), with permission.
change during the transformation from fifth instar to adult, although the sensitivity of Type III receptors (subgenual; Küehne, 1982) increases by approximately 10dB. The projections of vibratory and auditory receptors do not overlap. Halex et al. (1988) state that the projection areas of auditory receptors in the pro-, meso- and metathoracic ganglia are developed by the third instar. At this stage, some fibres already project to the suboesophageal ganglion.

8.4.3 Comparative aspects of cricket and grasshopper auditory projections

Although auditory receptors of both locusts and bushcrickets project to mVAC, there are clear differences in the organisation of this neuropil. Most importantly, there is a tonotopic arrangement of the auditory afferents in bushcrickets, but not in locusts. In the latter species, the arrangement is primarily somatotopic, in that it reflects the peripheral segregation of the receptors into four groups. This raises two relevant points. First, the projections of afferents from within any particular receptor group of the locust have not been thoroughly studied, presumably because of the difficulty of tracing both the central and peripheral branches of a neuron recorded intracellularly. It is possible, therefore, that tonotopic mapping is present, but on a scale that renders it undetectable using current techniques. The second point is one of terminology, for although great emphasis has been placed on the tonotopic nature of the mapping in bushcrickets, it should be realised that this is also a somatotopic map. This is so because the receptor cells form an ordered array in the periphery, in which position along the tympanum is correlated with frequency response. The observed mapping in the CNS may therefore appear tonotopic to a neurophysiologist, but to a developmental biologist, the spatial relationships between the receptors may be far more important.

A second difference between bushcrickets and locusts is that in the former, the projections of auditory afferents are found throughout mVAC, whereas in locusts they are restricted to the posterior ventral region of mVAC. Römer et al. (1988) suggest that the apparently simpler wiring of the bushcricket auditory neuropil is appropriate for rapid avoidance responses to the calls of predatory bats, whereas grasshoppers and locusts require more complex processing to detect the lizards and birds that are their main predators. Relationships
between the structure of mVAC and the stridulatory behaviour of acridids and bushcrickets is an important area that requires further investigation.

A third difference, which now appears more important than had been supposed, is the occurrence of auditory projections into at least five neuromeres in the locust (abdominal 1, meta-, meso-, prothoracic and suboesophageal). Previously it had been supposed that each auditory afferent projected into all these neuromeres, but this is not the case (see Section 8.4.2). High frequency afferents project further anterior than do low frequency afferents. What is now required is a clarification of exactly which neurons project to each ganglion so that a functional explanation can be put forward for this important observation. It is already clear that high frequency afferents have the potential to pass their information directly to postsynaptic neurons in a larger number of ganglia than do low frequency afferents.

8.4.4 Auditory projections in other insects

The central projections of primary auditory afferents in Mantidae and Noctuidae have been summarised by Boyan (1993), who points out the clear developmental homologies revealed by Meier and Reichert (1990) and Meier et al. (1991). Boyan’s (1993) review thus updates the earlier one of Michelsen and Larsen (1985) in this respect. Briefly, in these insects, as in bushcrickets and locusts, the auditory receptors project principally ipsilaterally. Despite the varying degrees of ganglionic fusion, the afferents project in equivalent fibre tracts and commisures, and branch mainly in equivalent neuropils (i.e. dVCLII, mVAC, SMC; see Boyan et al. 1990 for the ganglionic structure).

In the cicada Cystosoma saundersii (Homoptera) also, the auditory afferents project ipsilaterally (Wholers et al. 1979; Doolan and Young, 1981; see Michelsen and Larsen, 1985), to form an “intermediate neuropil” in a position that appears similar to mVAC in Orthoptera. This conclusion must remain tentative, however, until individual auditory afferents can be stained and shown to project in clearly homologous areas as assessed in appropriate sagittal and transverse sections.

The vibration sensitive subgenual organ of the green lacewing, Chrysoperla carnea (Neuroptera) has been the subject of recent study (Devetak and Pabst, 1994), but its projections and those of the auditory organ in the wing (Miller, 1970) are as yet unknown.

Boyan (1993) compares the projections of auditory receptors in tympanate insects with those of the non-tympanate Dipteran Drosophila. Until very recently, Diptera were not known to possess external tympani. Robert et al. (1994) show, however, that the parasitoid fly Ormia ochracea does possesses an external ear located on the front face of the prosternum and, moreover, that this organ contains scolophorus receptor neurons arranged into a bulba acustica (see Section 3.3.1).

The receptor axons enter the fused thoracic ganglion complex through the
frontal nerve and project ipsilaterally into all three thoracic neuromeres, appearing densest in the prothoracic (Fig. 26a). It is clear from the overall pattern of staining (from a backfill of the auditory nerve) that there is a compact, dense region of branching near the midline, and approximately medial in the longitudinal axis, of each neuromere. The dorso-ventral position is not shown, so it is not possible to determine if the regions of branching correspond to mVAC, although this seems likely. Robert et al. (1994) suggest that the ormiine auditory receptors are derived evolutionarily from a neck chordotonal organ such as that known to exist in drosophilid and muscoid flies (Hertweck, 1931; Hengstenberg, 1991). Here, the organ is involved in proprioceptive control of flight posture. The projections are ipsilateral, but Hengstenberg (1991) gives no further details. A study of these projections would provide excellent comparative material.

Neck chordotonal organs also occur in Acrididae (Bräunig et al. 1981; Hustert, 1978), so it may be possible to expand the comparison to include other insect groups. In the acridids, the most similar, and possibly homologous, receptor (aCO; Bräunig et al. 1981) projects to the pro-, meso- and metathoracic ganglia (Fig. 26b). The branches in the two anterior ganglia form discrete tufts of projections in mVAC, which appear remarkably similar to those of the ormiine fly auditory receptor. Notably, the prothoracic aCO does not have dorsolateral branches in LAC. In the metathoracic ganglion the branches are more distinctly bilateral, but still run within mVAC (Pflüger et al. 1988). This pattern of branching is somewhat different to that of aCOs in the meso- or metathoracic ganglia of locusts, which have additional projections in the abdominal neuromeres and, particularly for metathoracic aCO, in lateral regions of the metathoracic ganglion. It should be noted, however, that although thoracic chordotonal organs project to mVAC, their branches are restricted to the anterior part of the neuropil and do not overlap with auditory afferents from the ear. If the newly described auditory organ in a dipteran is evolutionarily derived from a homologue of aCO, then only minor remodelling of the neural circuitry should have been necessary for it to make appropriate connections within the CNS.

8.5 CENTRAL ORGANISATION OF OTHER CHORDOTONAL ORGAN AFFERENT NEURONS

The central projections of virtually all known ventral thoracic and proximal leg chordotonal organs of *Locusta migratoria* and *Schistocerca gregaria* have been described by Bräunig et al. (1981), Hustert (1978) and Pflüger et al. (1988). It is beyond the scope of this review to describe all these patterns. Instead, we list those that have been studied, and make some fairly general overall statements. The chordotonal organs listed in Table 2 have been stained by these authors with sufficient precision to allow their central projections to be unambiguously identified.
Chordotonal organs of the proximal leg joints (aCO, pCO, vCO, cCO, pjCO, ajCO, apCO; Fig. 6b) and abdominal segments (plCO) of locusts have afferents that generally project both intra- and intersegmentally, and which have both ipsilateral and contralateral branches (Pflüger et al. 1988). In contrast, chordotonal organ afferents of more distal segments of the leg (FeCO, crista acustica, intermediate organ, subgenual organ) project exclusively intrasegmentally and ipsilaterally.

8.5.1 Central projections of the locust femoral chordotonal organ

The femoral chordotonal organ (FeCO) has been the subject of extensive study because of its relatively large size, peripheral location and supposedly important role in controlling leg movements. The FeCOs of all the legs are divided into two separated groups of neurons (scoloparia) (see Section 3.5.3), although the degree of fusion differs. In the metathoracic leg the two scoloparia are merged together so that they cannot be backfilled separately. Furthermore, it is likely that the results of intracellular recordings (described below) are confined to sensory neurons of the proximal scoloparium, as these have larger somata and axons.

There are several developments in our understanding of locust and stick insect FeCO projections that post-date the summary given by Pflüger et al. (1988). First, the projections of receptors in the two scoloparia of pro- and mesothoracic FeCOs of the locust are now clearly assigned to two different regions of central projections (Field and Pflüger, 1989). Second, there are differences in the central projections of individual afferent neurons from within one scoloparium of the metathoracic femoral chordotonal organ in the locust (Matheson, 1992a). Third, some aspects of the development are now described (Lakes-Harlan and Pollack, 1993; see Section 10.5.3). Fourth, the projections of individual afferent neurons from within one scoloparium of the metathoracic femoral chordotonal organ in the locust (Matheson, 1992a). Fifth, some aspects of the development are now described (Schmitz et al. 1991). Fifth, locust femoral chordotonal organ afferents have been shown to receive GABAergic input synapses on their central branches (Watson et al. 1993). In addition, the projections of FeCO afferents have been reported for the cockroach Periplaneta americana (Collin, 1985) and the fly Phormia regina (Merritt and Murphey, 1992).

Axons of the locust metathoracic FeCO enter the ganglion through nerve 5 (Pflüger et al. 1988) (see Fig. 27a), before immediately giving off anterior and posterior branches into aLAC and pLAC, some of which terminate dorsal to DCIII. The main neurites continue antero-medially, giving off branches into surrounding areas. There are two prominent medial bundles of branches, one entering DCIII and the other entering DCIV. There are no branches in vVAC or mVAC. All the branches remain ipsilateral, and none leave the metathoracic neuromere.

Studies of the metathoracic femoral chordotonal organ of Locusta migratoria have shown that within the organ the somata are arranged so that
Fig. 27. Afferents from the femoral chordotonal organ of the locust project medially in two main bundles (a) which appear to correspond to those seen in the stick insect (d). In both insects there are also prominent lateral branches. Note that the metathoracic ganglion of the locust (a-c) contains three neuromeres (metathoracic, abdominal 1 and 2) merged together, hence its elongated shape as compared to that of the stick insect, which is a single neuromere (d-f). Some individual afferents physiologically characterised in the two species have similar central branching patterns (compare b with e, and c with f). As described in the text, however, others are apparently quite different. The neurons in b and e respond to negative accelerations of the receptor strand; those in c and f respond phasically and tonically to stretch of the receptor strand (tibial flexion). a, Burrows (1987b); b,c, Matheson (1992a); d, Schmitz et al. (1991); e,f, Ansgar Büschges (unpublished).

those with similar responses are grouped together (Matheson, 1990). Moreover, the central projections of some of the afferents with different responses are different (Matheson, 1992a). Burrows (1987b) first suggested, on the basis of whole nerve backfills (Fig. 27a), that different afferents of the locust metathoracic FeCO have different projections in the metathoracic ganglion. At that time, however, there were no physiological data to match up with the proposed morphological differences.
Matheson (1992a) subsequently recorded from these afferents at a site near the ganglion so that, once physiologically characterised, they could be filled with cobalt dye. This study indicated that neurons with different patterns of branching do, indeed, have different physiological responses. A shortcoming of the method was that the branches were analysed in only two dimensions (i.e. viewed from ventral), so much information was lost.

All metathoracic FeCO neurons recorded by Matheson (1992a) have a main neurite that extends as far anterior as DCIII, and all except some position-and-acceleration receptors have branches in the dorsal lateral neuropil (aLAC, pLAC). Extension sensitive neurons, and acceleration sensitive afferents that fire at the start of extension movements, have branches extending towards both DCIII and DCIV (Fig. 27b), whereas flexion sensitive afferents generally lack the branch near DCIV (e.g. Fig. 27c). For extension sensitive neurons at least, the length of the medial branches (i.e. their nearness to the midline) is correlated with the femoro-tibial angle that elicits maximal phasic or tonic firing. Extension sensitive neurons that are most sensitive to positions or movements near 120° (i.e. at extended angles) have medial branches that terminate nearer the midline than do those of afferents that respond most strongly near 0°. Two groups of neurons have very restricted regions of branching. Neurons that respond to flexion velocity across the full range of femoro-tibial angles and fire tonically at all leg angles (i.e. have a weak position dependence) have branches in the dorsolateral neuropils but no other large branches off their main neurite. Neurons with a strong position response and a response to acceleration have no large branches of the main neurite. These results differ somewhat from those for the stick insect (A. Büschges, pers comm; see Section 8.5.2).

Pflüger et al. (1988) also describe the projections of locust pro- and mesothoracic FeCOs, from whole-nerve backfills. Until recently, the two scoloparia of the locust pro- or mesothoracic FeCOs had not been stained individually, although fills of the whole organ (Pflüger et al. 1988) revealed two distinct populations of fibres. Field and Pflüger (1989) subsequently showed in the locust that the two groups are the projections of the two scoloparia (Fig. 26e). Neurons from the distal scoloparium have dorsolateral branches in aLAC and pLAC. The main neurites continue anterio-medially, giving off branches along their length, before terminating in two distinct bundles, one between DIT and VIT, at the level of DCIII, and the other between VIT and VMT, at the level of DCIV/SMC (Fig. 26ei). The comparable branches of the metathoracic FeCO enter DCIII and DCIV, respectively. None of the branches enter mVAC or vVAC, cross the midline or leave the ganglion. In contrast, neurons from the proximal scoloparium, which is apparently vibration-sensitive (see Section 3.5.3), form a dense cluster of branches in mVAC, with few lateral branches. Some axons from this scoloparium cross the midline, but not by more than 30 µm (Fig. 26eii).
8.5.2 Central projections of the stick insect femoral chordotonal organ

The anatomy of the thoracic ganglia of the stick insect *Carausius morosus* has been described by Kittmann *et al.* (1991). Although the terms used for some of the prominent features differ from those used in the locust literature (cf. Pflüger *et al.* 1988), the overall ganglionic structure is very similar. The FeCOs of stick insect legs are each divided into two distinct scoloparia. Physiological evidence (Kittmann and Schmitz, 1992; Büschges, 1994) indicates that it is the ventral scoloparium alone that mediates reflex control of the tibia (the function of the dorsal scoloparium remains unknown).

The projections of the entire FeCO (both scoloparia) reveal an overall pattern of branching similar to that seen for the distal scoloparium of the locust pro- and mesothoracic FeCOs (Schmitz *et al.* 1991) (Fig. 27d). Immediately after the neurites enter the ganglion, some neurites appear to branch anteriorly and posteriorly, giving rise to branches in the lateral dorsal neuropils (rdl, cdl = aLAC, pLAC). A central group of fibres projects anterio-medially from the nerve (ncr = n5) before bifurcating to form two distinct medial bundles. The anterior of these bundles enters DCIII whereas the posterior bundle projects slightly further medial, at the level of DCIV, and is ventral to VIT.

Schmitz *et al.* (1991) were unable to stain the two scoloparia independently, but speculate that the projections may be distinct. They tentatively suggest that the central bundle of fibres that bifurcates medially to give rise to the medial bundles at the level of DCIII and DCIV is distinct from the population of fibres that bifurcate laterally to give rise to the dorsolateral branches. Comparison with subsequent intracellular fills of stick insect FeCO afferents (Büschges, 1994), and those of the locust metathoracic FeCO suggest that this conclusion may be premature. For instance, both of the ventral scoloparium neurons illustrated by Büschges (1994) seem to have both a central bifurcating branch, and an anterior branch near the lateral margin of the neuropil (also see Fig. 27f). Similar neurons, and others with more distinct anterior and posterior lateral branches, are common in the locust metathoracic FeCO (Matheson, 1992a), so this pattern seems to be typical of FeCO afferents that are involved in proprioceptive reflexes. There are no published stains of FeCO afferents with the required alternative morphology (i.e. dorso-lateral branches and no “central” neurite). Burrows (1987b) illustrates two metathoracic FeCO neurons (from *Schistocerca gregaria*) that fit this description, but they were drawn from a nerve backfill, and almost certainly represent partially filled cells (see Matheson, 1992a). Clearly, further evidence is required to substantiate the two proposed configurations in the stick insect.

Ansgar Büschges (pers comm) notes that position sensitive FeCO neurons of the stick insect send prominent branches anterior and posterior to the main neurite (Fig. 27f), whereas velocity or acceleration sensitive neurons have only short branches that remain near the main neurite
(Fig. 27e). Most position sensitive neurons have a lateral branch in the dorsal neuropil, anterior to the neurite. Additionally, the branches of position sensitive neurons end relatively laterally, at the level of the ipsilateral connective, whereas the branches of velocity or acceleration sensitive afferents extend closer to the midline. Some specific neurons in the locust *Locusta migratoria* and the stick insect *Carausius morosus* appear to have similar central projections (compare Fig. 27b with 28e, and 28c with 28f). In many other cases, however, there are marked differences. Other aspects of FeCO structure and function appear well conserved in the two species, so this apparent discrepancy should be investigated in more detail. Differences in the equipment used to stimulate (and therefore characterise) the afferent neurons of the two species may have contributed to the apparent difference. For example, Matheson (1992b) was not able to directly test acceleration sensitivity, and instead relied on several indirect criteria to infer this component of the response. Even if this is the case, the results remain contradictory. For instance, Matheson reports that in the locust, neurons responsive to acceleration but only weakly responsive to position have widespread branches, including some extending medially towards DCIII. In contrast, neurons that respond well to both acceleration and position have no large branches off the main neurite, which terminates at the level of the ipsilateral connective. They have no branches at all in the lateral dorsal neuropils.

### 8.5.3 FeCO central projections in the cockroach and fly

Collin (1985) illustrates the central projections of many mechanoreceptor afferents of the cockroach leg, including the metathoracic femoral chordotonal organ. The projections, determined from peripheral cobalt backfills, are restricted to the ipsilateral side of the ganglion and appear to form two groups. In these two respects, they resemble those of the stick insect and locust (although in the cockroach there are no morphological grounds to suppose that the two groups of fibres represent afferents from two peripheral scoloparia). Here the resemblance ends. In other respects, the projections are markedly different. First, only 15 axons were stained (six anterior and nine posterior in nerve 5). Second, there are relatively few branches in the dorsal lateral neuropils, but apparently more in the “ventral neuropil”. Third, the two distinctive parallel bundles of fibres that, in locust and stick insect, run towards DCIII and DCIV are absent. Instead, there is a dorsal, anterior-posterior band of fine branches running along the midline. Murrain and Ritzmann’s (1988) illustration of the same projections is quite different. The afferents branch over a much more restricted area of the ganglion (apparently confined primarily to a medial ventral part of the ganglion, with fewer branches in the dorsolateral and lateral neuropils). It is possible that most of the afferents stained by the latter authors represent homologues of afferents in the locust proximal scoloparium, which project to mVAC. In light of these profound differences
and inconsistencies, the results from the cockroach should be treated with great caution until confirmed.

Merritt and Murphey (1992) summarise unpublished data showing central projections of the prothoracic femoral chordotonal organ of the fly. The projection is entirely within mVAC, suggesting that the stained afferents are vibration sensitive. There are apparently no FeCO afferents with branches in the dorsolateral neuropil, suggesting either that this type does not exist in the fly, or that only one scoloparium was stained.

8.5.4 Central projections of tarsal and cercal chordotonal organs

The mesothoracic tibiotarsal CO has projections in lateral and lateral dorsal regions of the mesothoracic ganglion (Laurent, 1987a). They are exclusively ipsilateral (Fig. 26c). It is interesting to compare these projections with those of the subgenual organ (Mücke and Lakes-Harlan, 1995), the proximal scoloparium of the femoral chordotonal organ (Field and Pflüger, 1989) (Fig. 26c-e), and chordotonal organs at the most proximal thoraco-coxal joint. Afferents of the most distal organ (the tibio-tarsal CO) do not project as far medial in the ganglion as those of the others. Its most medial branch terminates approximately 175 µm from the midline. The most medial branch of the next most distal receptor, the subgenual organ (whose somata are located in the proximal tibia) extends to within 60 µm, whereas that of the more proximal femoral CO terminates within 32 µm of the midline. The overall projection patterns of the most proximal coxal or thoracic chordotonal organs are quite different in shape, but it is notable that they often branch as far medial as the midline, or even have contralateral projections (e.g. Fig. 26b). Bräunig et al. (1981) have already noted for the coxal and thoracic chordotonal organs that the more distal the organ, the weaker is the tendency for its afferents to project into adjacent ganglia, and the more likely it is to have branches in the dorsolateral neuropil.

Trichoid hairs on the legs of the locust Schistocerca gregaria are also arranged into a somatotopic map, in which the three axes of the leg are represented within the CNS in a three dimensional projection area (Newland, 1990; Mücke and Lakes-Harlan, 1995). Here also, afferents from hairs that are more distal on the leg project more laterally within the ganglion, although the hair afferents project within VAC and not in the areas that contain chordotonal organ projections. This similarity of organisation in different neuropils of the ganglion suggests a strong influence of receptor position on the projection area.

The cercal chordotonal organ of the cockroach Periplaneta americana projects entirely within the ipsilateral half of the terminal abdominal ganglion, where it forms three distinct areas of branching (Füller et al. 1981). One of the groups projects more ventrally than the others, but this has not been assessed in transverse sections, so it is not possible to determine what neuropils contain
the terminal branches. It is surprising that this chordotonal organ, which lies close to the midline of the animal, has no intersegmental projections (see Section 8.5). One possibility is that the lateral group of fibres, which follow a fairly straight, unbranched course anteriorly through the ganglion (Füller et al. 1981), represent partially filled ascending axons.

To our knowledge, projections of the following chordotonal organs have not been clearly described in any insect: those of the mouthparts and antennae, including Johnston’s organ; the ovipositor; and the tegulae (the latter have been stained together with other receptors by Tyrer and Altman (1974) and Büschges et al. (1992)).

9. Processing of information from chordotonal organs

The processing of information from auditory chordotonal organs forms a huge field that is beyond the scope of this review. Readers are directed to reviews by Schildberger et al. (1989), Huber et al. (1990), Schildberger (1994), Michelsen (1994), Hoy (1994) and Römer (1994).

The most thoroughly studied proprioceptive pathways in insects are probably those of the femoral chordotonal organs of the locust and stick insect. In the locust, the approach has primarily been to use intracellular recordings to investigate neuronal activity during behaviour. In the stick insect, the approach has often been to characterise the properties of the control system in engineering terms. Each method has its advantages and shortcomings, but together they provide a great deal of valuable information about the basic principles of neural action that underlie the control of posture and movement. In this review we will attempt to synthesise what is known about both locust and stick insect femoral chordotonal organ control systems. The processing of information from other chordotonal organs will then be dealt with in light of this large body of knowledge.

9.1 Femoro-tibial chordotonal organ

9.1.1 Neurotransmitters

All known direct effects of femoral chordotonal organ afferents are excitatory (e.g. Burrows, 1987b; Burrows et al. 1988), suggesting that these neurons contain an excitatory neurotransmitter. Lutz and Tyrer (1988) report that approximately 30 afferent neurons from the locust (Schistocerca gregaria) femoral chordotonal organ are immunoreactive to choline acetyltransferase antiserum, and 30 to 40 are immunoreactive to 5-hydroxytryptamine antiserum. At the time of this work it was thought that the femoral chordotonal organ contained at most 55 neurons (see Section 3.5.3), so the tentative conclusion was that some neurons must therefore be immunoreactive to both substances.
Later work (Matheson and Field, 1990) showed that the FeCO contains approximately 90 neurons, so this conclusion is considerably weakened. A recent attempt (Watkins et al. 1995) to investigate further the presence of different neurotransmitters within the FeCO of Schistocerca gregaria failed to reveal any 5-hydroxytryptamine immunoreactivity within the organ, thus contradicting the earlier results. It therefore appears that afferent neurons of the locust FeCO do not contain 5-hydroxytryptamine.

In Drosophila melanogaster, histamine is found in the photoreceptors and in movement-sensitive hair sensilla, but not in chordotonal organs (Buchner et al. 1993).

9.1.2 Central circuitry
Afferents from the femoral chordotonal organ in both locusts and stick insects project entirely ipsilaterally within their segmental ganglion (see Section 8.5.1-2). This means that their direct output effects must also be restricted to this region. Local circuits underlying the processing of proprioceptive and exteroceptive information, and the control of motor neurons, have been reviewed by Burrows (e.g. 1985a, 1989, 1992, 1994), with emphasis being placed on the processing of exteroceptive inputs from tactile hairs of the locust. Nevertheless, subsets of the same populations of motor neurons and interneurons are involved in processing femoral chordotonal organ information, so these reviews provide an essential background. We consider only pathways that have been shown to carry proprioceptive information, but mention comparable exteroceptive pathways where this can shed light on the known reflexes. Work on the stick insect has been reviewed by Bässler (1983, 1993).

Four general classes of neurons have been shown to receive direct inputs from locust femoral chordotonal organ afferents. These are: motor neurons, spiking local interneurons, non-spiking local interneurons and spiking intersegmental interneurons (Fig. 28a). Although the femoral chordotonal organ of the stick insect has clear effects on the first three classes, none of these effects have yet been shown to be mediated by direct connections.

The connections demonstrated for the locust are summarised below and in Fig. 29, forming the basis for a discussion of the reflex effects and processing of femoral chordotonal organ information in general.

9.1.2.1 Connections with motor neurons In the locust Schistocerca gregaria, some femoral chordotonal organ sensory neurons make connections with flexor tibiae motor neurons while others connect with the slow extensor tibiae motor neuron, but none connect with both extensors and flexors (Burrows, 1987b). A given afferent can excite several of the nine flexor motor neurons. Not all similar afferents make connections with a particular flexor tibiae motor neuron, so it is clear that sensory information is passed to specific targets by
Fig. 28. a. Positions of the somata of prominent groups of motor neurons and interneurons involved in the processing of information from the locust metathoracic femoral chordotonal organ. The neurons shown here have their branches in the right half of the ganglion, but equivalent counterparts are found contralaterally. Spiking local interneurons fall into three groups on the ventral surface: anterior medial, anterior lateral and midline. Note that the midline and anterior lateral groups have their somata contralateral to their branches. The somata of ascending intersegmental interneurons lie near the anterior medial group. Nonspiking interneurons (not shown) have somata scattered over much of the ventral surface of the ganglion. The fast and slow extensor tibiae motor neurons (FETi and SETi) lie more laterally; the somata of FETi being the largest in the ganglion. The nine flexor tibiae motor neurons lie in the shaded region anterior medial to FETi. Three common inhibitor motor neurons (Cl_{1-3}) lie closer to the midline. b. Morphology of the single identified nonspiking local interneuron named DCVII,1 in the locust by Wilson (1981). c. The companion neuron DCVII,2. d. A stick insect nonspiking local interneuron named E4 in the stick insect (Driesang and Büschges, 1993), having a branching pattern like that shown in b. e. Another stick insect nonspiking local interneuron named E4 by Büschges (1990), and proposed to be homologous to that shown in c. Data in a are from Nagayama (1989); Siegler and Burrows (1984), with permission; Watson et al. (1985), with permission.
different afferents. Some of the afferents appear to make direct connections with the fast extensor tibiae motor neuron (T. Matheson, unpublished observations). Possible inputs onto the common inhibitory motor neurons or onto motor neurons of other leg joints have not been assessed for monosynapticity.

9.1.2.2 Connections with spiking local interneurons Spiking interneurons of the ventral midline group (Fig. 28a; Siegler and Burrows, 1984) receive direct excitatory inputs from femoral chordotonal organ afferents (Burrows, 1987b). Individual afferents can synapse with both motor and interneurons, and several afferents can converge onto a particular interneuron. Burrows (1985b) suggested that some spiking local interneurons that receive direct inputs from femoral chordotonal organ afferents may also receive direct inputs from tactile hair afferents. More recently, however, this interpretation has been questioned, although not overturned (Burrows and Newland, 1993). It seems most likely that separate populations of midline spiking local interneurons are responsible for processing these two types of sensory information. Interneurons that receive femoral chordotonal organ inputs lack branches in the most ventral regions of the neuropil (aVAC, lVAC, vVAC) that contain the tactile hair afferents. Instead, they branch in aLAC and pLAC, to where the femoral chordotonal organ afferents also project (Burrows and Newland, 1993).

It is not known if femoral chordotonal organ afferents synapse directly with spiking local interneurons of the anterior medial or antero-lateral groups (Fig. 29).

9.1.2.3 Connections with non-spiking local interneurons Burrows et al. (1988) show that femoral chordotonal organ afferents connect monosynaptically with particular non-spiking local interneurons. Here, as elsewhere, the effect is excitatory (Fig. 29). There are also longer latency inhibitory effects, apparently mediated by spiking local interneurons. The nonspiking interneurons with inputs from the femoral chordotonal organ have a variety of branching patterns, and their somata are in different locations within the ganglion (Watkins et al. 1985), but at least some of their branches overlap with those of the afferents (Burrows et al. 1988).
Fig. 29. Summary of the pathways known to process femoral chordotonal organ information in the locust. Each large circle represents a population of neurons, and each solid line a known direct connection between neurons. Not all neurons in a given population make all the illustrated connections. Dashed lines indicate pathways that have not been fully characterised. Question marks indicate pathways that exist for exteroceptive afferents, but which have not been demonstrated for chordotonal organ afferents. Numbers refer to papers that describe each pathway. Intersegmental, spiking and nonspiking interneurons are distinguished by different patterns of stippling.
9.1.2.4 Connections with intersegmental interneurons  Mesothoracic descending interneurons are excited by movements of both the tibio-tarsal and femoro-tibial joints (Laurent, 1986, 1987a,b,c). There are direct connections between the tibio-tarsal chordotonal organ afferents and the intersegmental interneurons (Laurent, 1987a), but possible inputs from femoral chordotonal organ afferents have not been assessed (Fig. 29). Büschges (1989) shows that a wide variety of intersegmental interneurons of the stick insect are influenced by the FeCO, but it is not known if the effects are mediated by monosynaptic connections. Many of these interneurones have both ascending and descending axons, and not all have their somata in the mesothoracic ganglion whose FeCO was stimulated. The interneurons respond to position, velocity or acceleration of the FeCO, in a range of combinations tabulated by Büschges (1989). Some interneurons are inhibited by particular movements, so these pathways are likely to be polysynaptic. Responses to acceleration are always excitatory, whereas those to velocity can be either excitatory or inhibitory.

Ascending intersegmental interneurons with somata in the metathoracic ganglion are known to receive excitatory inputs from femoral chordotonal organ afferents (Laurent and Burrows, 1988a). Individual interneurons can be excited by one direction of tibial movement, or by both directions. In the latter case, it is not clear if both extension-sensitive and flexion-sensitive afferents make direct connections with the interneuron, or if one of the pathways is indirect. Other interneurons of this population are inhibited by one direction of tibial movement, but the pathway is indirect (via the midline spiking interneurons; Fig. 29).

Intersegmental interneurons that receive direct inputs from femoral chordotonal organ afferents do not have branches in the ventralmost regions of neuropil (aVAC, IVAC, vVAC) but, instead, have branches primarily in aLAC and pLAC, which also contain the projections of the afferents. These interneurons apparently do not process information from tactile hairs (Laurent and Burrows, 1988a).
In the cockroach, femoral chordotonal organ afferents have weak effects on some members of a group of intraganglionic interneurons named dorsal posterior group (DPG) interneurons (Ritzmann and Pollack, 1986; Murrain and Ritzmann, 1988). The DPG interneurons are individually identifiable, and are known to be interposed between ventral giant interneurons and motor neurons. They are likely to be involved in escape behaviour. The effects of femoral chordotonal organ stimulation are weak, however, and there is no evidence that they are direct.

9.1.2.5 Properties of central neuronal pathways

The various populations of interneurons that receive direct inputs from femoral chordotonal organ afferents also influence each other. The specificity of these connections within and between populations provides many parallel pathways for information flow and must, therefore, be an important means by which behavioural flexibility is achieved. The simplest polysynaptic pathways involve a single spiking or non-spiking interneuron, which then makes output connections with leg motor neurons (Fig. 29). All known outputs of the midline spiking local interneurons are inhibitory, so disynaptic pathways involving these interneurons enable femoral chordotonal organ afferents to exert fairly short latency, reliable, inhibitory effects on motor neurons (Burrows and Siegler, 1982). The local non-spiking interneurons are also known to exert strong excitatory or inhibitory effects on leg motor neurons, but it has proved difficult to determine if these effects are mediated by monosynaptic connections (Burrows and Siegler, 1976, 1978). Burrows (1989) shows that a given non-spiking interneuron can simultaneously depolarise one motor neuron and hyperpolarise another. Only the hyperpolarisation, however, can be attributed to a direct effect (Fig. 29). The depolarisation must occur as a result of disinhibition through a more complex pathway.

Longer polysynaptic pathways include inhibitory synapses from the midline spiking interneurons onto the non-spiking interneurons (Burrows, 1987a, Burrows et al. 1988), descending interneurons (Laurent, 1987a, 1988) and between non-spiking interneurons (Burrows, 1979).

Midline spiking local interneurons that process exteroceptive inputs make direct inhibitory connections with antero-medial spiking local interneurons (Nagayama and Burrows, 1990), but it is not known if there is a similar pathway for proprioceptive information. Mesothoracic midline spiking interneurons that process proprioceptive information do, however, connect directly with descending intersegmental interneurons (Laurent, 1987c), as do those that process exteroceptive information.

A final pathway that should be considered involves the outputs of mesothoracic descending interneurons. Their inputs in the mesothoracic ganglion come from both exteroceptors and proprioceptors (see Section 9.1.2.4). In turn, they make either excitatory or inhibitory output connections with local non-spiking interneurons and motor neurons of the metathoracic ganglion, but not with the midline spiking local interneurons (Laurent and Burrows, 1989a,b). This means that there must be at least two distinct
subpopulations but, as yet, it is not known if these two groups have different inputs.

In the locust, but not in the stick insect, there is a direct central connection from the metathoracic fast extensor tibiae (FETi) motor neuron onto all the known flexor tibiae motor neurons (Hoyle and Burrows, 1973; Burrows et al. 1989). There is no equivalent connection in the prothoracic or mesothoracic ganglia of the locust. Spikes in FETi depolarise the ipsilateral flexors, causing some to fire at high frequency.

Motor neurons controlling the legs do not always act as simple followers of their inputs. For example, the fast coxal depressor motor neuron of the cockroach *Periplaneta americana* can exhibit plateau potentials under some circumstances (Hancox and Pitman, 1992, 1993). These plateau are sufficient to cause the motor neuron to spike (Hancox and Pitman, 1991), and can be elicited by synaptic inputs from the contralateral nerve 5 (Hancox and Pitman, 1993). The pathway underlying this contralateral effect is unknown, and must involve several synapses. Locust flight motor neurons are also able to generate such plateau potentials (Ramirez and Pearson, 1991), but it is not known if any other motor neurons are able to do so, or under what natural conditions this property becomes important. The membranes of non-spiking local interneurons in the locust *Schistocerca americana* show voltage-dependent non-linearities that influence the effect of synaptic inputs on the transmembrane voltage (Laurent, 1990, 1991; Laurent et al. 1993). In none of these cases has it been suggested that chordotonal organ inputs are important in driving the neurons into regions of membrane non-linearity, but it seems likely that they will do so when combined with other inputs impinging on the cells.

9.1.2.6 Homologies amongst non-spiking interneurons of different insects For some time, local non-spiking interneurons have been known to process information from the femoral chordotonal organ (see Section 9.1.2.3 and the many papers cited in reviews by Burrows, 1981, 1994; Siegler and Burrows, 1980; Wilson and Phillips, 1983). This early work described some of the pathways by which the information was received and passed on, and showed that altering the membrane potential of individual interneurons could affect the strength of reflexes mediated by the femoral chordotonal organ. More recent work by Büschges and colleagues has shown that comparable interneurons (e.g. Fig. 28b-e) exist in the stick insect (Bässler and Büschges, 1990; Büschges, 1990; Schmitz et al. 1991; Driesang and Büschges, 1993; Büschges et al. 1994; reviewed by Bässler, 1993). Nonspiking interneurons are known to be important points of convergence for many types of information, and they act to coordinate the actions of groups of motor neurons (e.g. Burrows, 1985a, 1994). Here we try to bring together the recent work on stick insects and locusts.

Are interneurons identifiable? In his 1985a review, Burrows suggested that it is pointless trying to ascribe functions to particular interneurons studied using intracellular recording techniques. The reasoning behind this was that a
“function” could change as the behavioural context changed. Similarly, ascribing a name to a recorded neuron could be interpreted to mean that it is a single identified individual (as are some sensory or motor neurons, and a very few interneurons so far [e.g. Wilson, 1981; Wilson and Phillips, 1982]). The approach advocated by Burrows was to emphasise underlying principles of operation, rather than specific details of every recorded neuron. With this approach, he suggested, sufficient additional morphological and physiological data should gradually be built up to permit more specific questions to be focused on appropriate neurons at a later date. In the ten years since Burrows’ review, we have learned more about the nonspiking interneurons, and some seem particularly amenable to repeated testing, perhaps allowing them to be investigated in considerable detail. There remain substantial problems, however, for the different studies of possibly homologous interneurons often do not characterise them in comparable ways that would allow such homologies to be proposed. Future studies should, where possible, characterise neurons using the same tests as have been performed previously. Little progress will be made on forming a more cohesive view of central processing pathways if each additional study cannot be placed in the context of what has gone before. Such confusion will lead to needless repetition of experiments simply to determine if the particular neuron of interest has been investigated in some other context. The problems of classifying and maintaining an accessible catalogue of described neurons or classes of neurons are formidable, and deserve wide discussion.

Identified non-spiking interneurons  Nonspiking interneurons that respond to tibial (and presumably femoral chordotonal organ) movements were first illustrated by Pearson and Fourtner (1975) for the cockroach. One “Type I” neuron (they suggest that there is only one, but with little evidence) excites anterior and posterior coxal levator motor neurons and tibial flexor motor neurons. It has little effect on coxal depressors. Burrows and Siegler (1976) and Siegler and Burrows (1979) subsequently illustrated a neuron with similar morphology in the metathoracic ganglion of the locust, which also excites (fast) flexor tibiae and coxal motor neurons, and responds to imposed movements of the tibia. Similar neurons with the same pattern of inputs and outputs, but with an additional ipsilateral branch were also described by Siegler and Burrows (1979). Wilson (1981) later showed that, in the mesothoracic ganglion of the locust, there are three neurons with this similar morphology that can be individually identified on the basis of their branching and physiological properties. Only one has the ipsilateral branch. This he termed DC VII,1 (Fig. 28b). Of the other two, one (DC VII,2) (Fig. 28c) excites FETi and SETi, whereas the other (DC VII,3) excites flexor tibiae motor neurons, the levator trochanteris and coxal motor neurons. It seems probable, therefore, that DC VII,3 is the neuron illustrated by Pearson and Fourtner (1975) and Burrows and Siegler, 1976).

Non-spiking interneurons in the stick insect have more recently been named
according to their output effects on the tibial extensor and flexor muscles. For instance, Büschges (1990) describes six such neurons that excite the tibial extensor motor neurons, which he names E1-E6. Two interneurons that inhibit these same motor neurons are called I1 and I2. Other possible output targets of these interneurons are not known, however, so the naming scheme should not be used to infer anything more about the properties or role in reflexes of these neurons. Interneuron E4 receives direct input from afferents of the FeCO (Sauer et al. 1995) as do interneurons E2-E6 (A. Büschges pers comm).

One type of nonspiking interneuron in the mesothoracic ganglion of the stick insect (Type E4, Büschges, 1990) (Fig. 28e) was initially reported to correspond to the identified neuron named DC VII,2 by Wilson (1981). Later, however, Driesang and Büschges (1993) and Büschges et al. (1994) state that in the stick insect there are at least two neurons in each hemiganglion that fit the description of E4, but in the former paper they illustrate a neuron with morphology more like that of DC VII,1 (it has a prominent region of branches ipsilateral to its soma; Fig. 28d). Ansgar Büschges (pers. comm.) confirms that there are at least two E4 neurons, probably with different branching patterns. In one preparation both were stained, and one had the region of ipsilateral branches while the other did not. In the locust, at least, the interneuron with ipsilateral branches (DC VII,1) excites the flexor tibiae motor neurons and not the extensors. The situation must differ in some way in the stick insect, because neurons with both morphologies are termed E4 and both excite extensor tibiae motor neurons. This difference between the genera is not mentioned by Büschges and Wolf (1995) or Wolf and Büschges (1995) who treat the interneurons with and without contralateral branching as equivalent in both locust and stick insect. It seems likely that in both species the neuron with ipsilateral branches has some input or output connections that are not found in that with solely contralateral branches. Bearing in mind that the term E4 most likely refers to at least two distinct neurons in the stick insect, it is now appropriate to consider the additional data that studies of the stick insect have provided. Type E4 interneurons are the most thoroughly studied, so they will be used as a starting point before briefly discussing other nonspiking interneurons described in the stick insect.

Processing of femoral chordotonal organ information by non-spiking interneurons of Type E4

Studies in the locust have shown that non-spiking interneurons respond to movements of the tibia, and, more specifically to the activity of individual femoral chordotonal organ afferents (Burrows et al. 1988). This does not preclude the involvement of other receptors, but it does indicate the importance of the femoral chordotonal organ in leg control pathways.

Interneurons of Type E4 are depolarised by both stretch (tibial flexion) and relaxation (tibial extension) of the femoral chordotonal organ apodeme (Büschges, 1990). They are more sensitive to flexion. The effects in the interneurons are seen about 5ms after the start of the movement so, taking
conduction time into account, they appear to be direct or mediated by a short pathway, but this has not been confirmed by other techniques. The amplitude of depolarisation is velocity-, but not position-dependent. This is interesting, because Matheson (1990, 1992b) shows that most of the velocity-sensitive afferents of the locust are position dependent: only a few have equal responses at all tibial angles. The implication is that either (1) E4 interneurons receive information from an array of position-dependent velocity receptors that together span the range of leg angles (range fractionation) or (2) that they receive inputs primarily from the position-independent velocity receptors. This latter group are amongst the most sensitive, responding to movements as slow as 0.5°s⁻¹ (Matheson, 1992b).

An important question that remains to be answered for the processing of femoral chordotonal organ information is how that from the different classes of afferent is distributed onto the different types of motor and interneurons. There is, at least, convergence of information from extension-velocity-sensitive and flexion-velocity-sensitive afferents onto E4 interneurons, because no afferents respond to both signs of velocity. Laurent and Burrows (1988b) illustrate a neuron in the locust mesothoracic ganglion with the same morphology as E4 (DC VII,2), which has direct excitatory inputs from tactile hairs on the tarsus, and outputs onto the levator tarsi motor neuron. It is not further characterised. Büschges et al. (1994) show that tactile stimulation of the tarsus of any ipsilateral leg of the stick insect causes a depolarisation of the mesothoracic E4 interneurons, and although it is not known if the pathway is direct, the depolarisation always precedes motor neuronal activity that drives compensatory leg placement movements. Interestingly, the mesothoracic E4 is depolarised during both lifting and lowering compensatory movements of the mesothoracic leg. Clearly, therefore, E4 interneurons alone are not sufficient to explain the compensatory behaviour, although they may contribute to some aspects of it.

Artificial depolarisation of a Type E4 interneuron causes the SETi discharge rate to increase, and sometimes elicits spikes in FETi (Büschges, 1990). Hyperpolarisation from ‘rest’ has no effect on the SETi spike rate. Depolarisation of E4 interneurons enhances the reflex activation of SETi caused by movements of the femoral chordotonal organ apodeme, suggesting that these interneurons can influence the expression of leg reflexes. Type E4 interneurons also excite at least four protractor coxae motor neurons, in addition to their probable excitation of the levator tarsi. These neurons are therefore in a position to not only integrate a range of sensory signals of different modalities from different parts of the leg, but also to pass this information on to groups of motor neurons controlling the different leg joints, as already shown for nonspiking interneurons in the locust (Burrows, 1980). Clearly, this gives them the potential to participate in, and perhaps direct, complex reflex responses.

Further evidence that interneurons of Type E4 participate in leg reflexes comes from the study by Driesang and Büschges (1993). They show that
depolarisations of the membrane in response to movements of the femoral chordotonal organ apodeme have time-courses appropriate to drive the behaviour known as catalepsy in the stick insect. In catalepsy, stick insects move their legs back to an initial position only very slowly after they have been forcibly moved. The time taken for a depolarisation in interneuron E4 to decay can be explained by one exponential equation at low stimulus velocities, and by two exponential equations (one with a short time-course and the other with a longer time-course) at higher velocities. The component with the short time constant becomes more important as velocity increases. Neither of the time constants appears to be directly related to the time constant of the neural membrane, and it appears that it is the properties of the inputs, and not active membrane properties, that primarily determine the shape of the depolarisation. The time-course of decay in the depolarisation is approximately the same as the time-course of decay in the SETi firing frequency elicited by the same movements. What has not been determined, however, is the time-course of the response of velocity-sensitive afferents (or even of the overall femoral chordotonal organ response) to the same movements. This is an important point if we are to assess the role of the interneurons in transforming sensory information. One conclusion of Driesang and Büschges’ (1993) paper is that monosynaptic inputs known to exist between femoral chordotonal organ afferents and SETi (in the locust) are not necessary for the production of the observed decay of SETi firing frequency in the stick insect (since the interneuron should be able to drive this pattern of motor output directly). It is not claimed that the interneuron is sufficient to do this, and it may be impossible to establish whether this is so because it would require elimination of all direct afferent pathway without substantially altering the observed response; clearly a difficult or impossible task. An alternative explanation for the observed response of SETi is that the pattern of recruitment and response properties of the afferent neurons define the time-course of the overall afferent response, and that this time-course of activity is passed in parallel to both the interneurons and motor neurons.

Büschges et al. (1994) show that in both quiescent and actively moving stick insects, artificial depolarisation of Type E4 interneurons is able to influence the activity of leg motor neurons, indicating that at least some of the pathways described for the quiescent animal in previous studies are also effective in an active animal performing voluntary movements. During walking, the membrane potential of Type E4 interneurons is modulated in time with the step cycle, with the depolarisation of E4 interneurons at the appropriate time to contribute to the activation of protractor coxae and extensor tibiae motor neurons. What is required now is a clear demonstration of the relative importance of these interneurons. For instance, how does the step pattern (or underlying motor neuronal activity) change if the interneurons are held tonically hyperpolarised by injected current?

Büschges and Wolf (1995) compare various nonspiking interneurons of the locust Locusta migratoria and the stick insect Carausius morosus. They state
that although the tibial control systems of the two species have many similarities, that of the stick insect has a more marked velocity dependence than that of the locust. This difference is generated within the CNS, and is not a property of the afferent responses. It seems that the more pronounced velocity dependence in the stick insect is important for the generation of catalepsy; a behaviour not exhibited by the locust. Büschges and Wolf (1995) suggest two mechanisms that might account for the lack of velocity dependence in the locust. First, the presynaptic gain control mechanism (see Section 9.3) demonstrated by Burrows and Laurent (1993) and Burrows and Matheson (1994) in the locust femoral chordotonal organ may be stronger here than in the stick insect. This is clearly a testable hypothesis. Second, there may be a different balance between convergent inhibitory and excitatory pathways in the two species.

**Other interneurons** Non-spiking interneurons other than those of Type E4 are also influenced by the femoral chordotonal organ and contribute to the control of leg movements. Büschges (1990) describes a total of eight types (E1-E6, I1, I2) that are influenced by movements of the femoral chordotonal organ apodeme. With the exception of Type E4, none of these interneurons appears to correspond to published examples in the locust, suggesting either that the two genera have different arrays of interneurons or, more likely, that many interneurons that respond to FeCO movements have not been described in both animals. As in the locust (e.g. Burrows et al. 1988), different non-spiking interneurons have different patterns of response to movements of the femoral chordotonal organ. Some are depolarised by one direction of stimulation and inhibited by the other, whereas others are influenced by one direction of movement only. The nature of the output connections of the interneurons determines whether they act to assist or resist the imposed stimulus. Perhaps more interestingly, some interneurons are either hyperpolarised (Burrows et al. 1988) or depolarised (Büschges, 1990) by both directions of movement. They thus act in both assisting and resisting pathways, as emphasised by Büschges (1990). Furthermore, it is clear that because many interneurons are activated in parallel, any given movement produces activity in both assisting and resisting pathways feeding back onto the imposed joint movement. The resultant movement must, therefore, be the sum of these opposing inputs. A final point concerns the possibility that the responses of non-spiking interneurons change as the activity state of the animal changes. Bässler and Büschges (1990) show that of eight characterised interneurons E1-E6 and I1-I2 (Büschges, 1990), half (E1, E4, E5, E6) do not show marked changes in response as the stick insect changes from being inactive to being active. Interneurons of Types E3 and I2, however, do show distinct changes. In quiescent stick insects, interneurons of Type E3 are depolarised by stretch of the femoral chordotonal organ, but in active animals the same stimulus causes a clear hyperpolarisation. Interneurons of Type I2 are depolarised by stretch of the femoral chordotonal organ in both quiescent and active stick insects, but the response in the latter state is approximately four times larger in amplitude. The mechanisms underlying these changes in response are unknown.
The role of non-spiking interneurons in walking stick insects was initially investigated by Schmitz et al. (1991). The branching patterns of the interneurons studied are similar to those of Types E1 and E2 of Büschges (1990), but this is not to say that they are the same cells. Changes in the membrane potentials of some of these interneurons are correlated with alterations of the membrane potentials of coxal motor neurons, and artificially manipulating the membrane potential of a single interneuron is sufficient to alter the step pattern. The inputs recorded in the interneurons were presumably derived from both central and peripheral sources. More recently, Wolf and Büschges (1995) have recorded from non-spiking local interneurons in a walking locust (*Locusta migratoria*). They showed that activity of particular interneurons can either support or resist an ongoing leg movement in different circumstances. Both supporting and resisting interneurons are active during any movement, so it appears that movements are generated by the balance of conflicting inputs. Furthermore, some interneurons have a strong influence on leg movements, and appear to participate in the switch from stance to swing, whereas other interneurons have a weaker influence on this transition, and appear to be primarily involved in coordinating groups of motor neurons.

9.1.3 Modulation of local networks by centrally generated patterns

Connections other than those described above also drive these populations of neurons. In addition, it is likely that the strengths of the various pathways alter in different circumstances, perhaps under the influence of circulating neuromodulators or hormones. In the locust, it is already known that a centrally generated rhythm, perhaps underlying walking, impinges on the midline population of spiking local interneurons (Wolf and Laurent, 1994). The rhythmic central inputs affect interneurons that process exteroceptive, proprioceptive or both types of sensory input. In addition, it could come from either an ipsilateral or a contralateral rhythm generator. These results clearly imply that the local processing of sensory information can be gated or modulated at this level during walking (and presumably during other behaviours as well). Wolf and Burrows (1995) show that femoral chordotonal organ afferent neurons receive presynaptic inhibitory inputs from a central pattern generator. It is already known that sensory feedback from movements of one leg can cause such inputs in afferents of an adjacent leg (T. Matheson, pers. obs. and see Wolf and Burrows, 1995) so, during walking, sensory and central inputs should both contribute to phase-dependent presynaptic inhibition of the afferents themselves.

9.1.4 Local reflexes mediated by the femoral chordotonal organ

9.1.4.1 Resistance reflexes In quiescent locusts, bushcrickets stick insects and cockroaches, the predominant effect of stimulation of the FeCO is a
resistance reflex (Schistocerca gregaria, Field and Burrows, 1982; Decticus albifrons, Theophilidis 1986b; Carausius morosus, Bässler, 1976; Periplaneta americana, Brodfuehrer and Fourtner, 1983). For example, relaxation of the femoral chordotonal organ (simulating tibial extension) excites tibial flexor motor neurons (predominantly through the direct connections between the afferents and motor neurons described above for the locust), and inhibits the antagonistic extensor tibiae motor neurons (through polysynaptic pathways). The resistance reflexes to imposed extensions and flexions of the tibia are unequal in magnitude, responses to flexion being smaller (see Bässler, 1993).

The extensor tibiae muscle of the locust is innervated by one fast (FETi) and one slow (SETi) motor neuron, whereas the flexor muscle is innervated by three fast, three intermediate and three slow motor neurons. The responses of the different motor neurons to movement of the femoral chordotonal organ apodeme differ. SETi is depolarised most by frequencies of approximately 5Hz. Faster or slower movements have less effect. In contrast, FETi is depolarised most by the most rapid movements tested (20Hz; Field and Burrows, 1982). The situation is different in the stick insect. Here, both FETi and SETi are depolarised maximally by frequencies of approximately 1Hz (Bässler, 1983). In the locust, SETi responds to maintained changes of joint angle with prolonged changes in membrane potential, but FETi does not (Siegler, 1981). This difference is not evident for the fast and slow flexor motor neurons of the locust (Siegler, 1981), but in the stick insect, slow flexor motor neurons are depolarised most by slow extension movements, whereas fast motor neurons are depolarised most by more rapid movements (Debrodt and Bässler, 1990). In both stick insect and locust, fast motor neurons adapt to repetitive stimuli more rapidly than do their slow counterparts (Field and Burrows, 1982; Bässler, 1983). Interestingly, Debrodt and Bässler’s (1990) paper reports that the flexor motor neurons are nearly always depolarised during extension movements, but that flexion movements only sometimes cause a hyperpolarisation. One explanation for this may be that under these quiescent conditions, the polysynaptic inhibitory connections are relatively less powerful than the direct excitatory ones. The reflex loop thus acts here as a rectifier. Siegler (1981) and Field and Burrows (1982) indicated that some flexor motor neurons of the locust respond to both directions of movement with a depolarisation, an observation also made later by Zill (1985b) for active locusts (see Section 9.1.4.3). It therefore seems likely that some of the locusts tested by the former authors were also in an active state. Alternatively, this may be the usual mode of action in both quiescent and active locusts. A few flexor tibiae neurons of the stick insect also respond to flexion movements with a depolarisation (Debrodt and Bässler, 1990).

The overall strength of resistance reflexes in both extensor and flexor motor neurons is dependent on the set position of the joint before the imposed movement. For example, SETi of the locust is depolarised more by a flexion movement that begins at a relatively flexed angle than by one that begins at a more extended position (Field and Burrows, 1982). For flexor motor neurons
also, reflex effects are strongest at more flexed angles (Field and Burrows, 1982). The positiondependent nature of the resistance reflexes in both extensor and flexor muscles is evident at each stage of the reflex pathway, including afferents, non-spiking local interneurons and motor neurons (Field and Coles, 1994). The conclusion is that the position dependence results from the recruitment of progressively fewer phasic femoral chordotonal organ afferent neurons sensitive to movements at extended angles (e.g. Matheson, 1990). It functions to enhance resistance reflexes that counter lateral displacements of animals standing on horizontal surfaces, and vertical displacements of animals standing on vertical surfaces (Field and Coles, 1994).

Zill and Forman (1983) showed that in restrained locusts, reflex responses to imposed femoral chordotonal organ stretch (tibial flexion) are variable, although they generally resisted the apparent movement. Once the animal has been trained to extend its leg (using an aversive heat stimulus), the resistance reflexes become much stronger and more consistent. In free standing (untrained) locusts, femoral chordotonal organ stimulation (tibial flexion) consistently elicits resistance reflexes (Zill, 1987). Similarly, in locusts standing on a swaying vertical substratum, the flexor tibiae muscle is activated in a resistance reflex that could compensate for variations in load, but the receptor(s) underlying this response are unknown (Zill and Frazier, 1990). Theophilidis and Burns (1990) also investigated the production of leg resistance reflexes in locusts, but again, the sense organ(s) underlying them were not established.

The femoral chordotonal organs of the prothoracic and mesothoracic legs of the locust (and all those of the stick insect) are composed of two groups of sensilla (scoloparia). Field and Pflüger (1989) have shown that, for the mesothoracic leg of the locust at least, the resistance reflexes (and presumably other reflexes as described in Section 9.1.4.3) are mediated by the distal scoloparium alone. The proximal scoloparium seems to be more sensitive to vibration than tibial position or gross movement, and produces weaker “startle” responses.

In active stick insects, imposed movements of the femoral chordotonal organ can lead to resistance reflexes that serve to maintain a set velocity of leg movement (Weiland and Koch, 1987). During voluntary flexion, an additional stretch of the chordotonal organ (mimicking increased velocity) slows the ongoing movement. Conversely, imposed relaxation of the femoral chordotonal organ (mimicking reduced velocity of flexion) causes an increase in the velocity of the ongoing movement. During voluntary extension movements also, superimposed movements of the femoral chordotonal organ lead to reflexes that act to stabilise apparent tibial velocity.

If the apodeme of the femoral chordotonal organ is detached from its insertion on the tibia and reattatched near the flexor muscle apodeme, then tibial movements will cause an incorrect (reversed) sensory signal (i.e. tibial extension will cause the femoral chordotonal organ to signal an apparent tibial flexion). When this operation is carried out in locusts or stick insects (Bässler, 1967, 1979) the tibia remains in a fully
extended position. In contrast, simple ablation of the femoral chordotonal organ has little effect on normal leg movements or walking coordination. Interestingly, if the reversal operation is carried out on juvenile (third instar) locusts, then the abnormal behaviour persists throughout subsequent moults as long as the apodeme remains in the reversed position. If the apodeme of such an adult is then cut, the leg returns to its usual position and again participates in approximately normal walking movements (Bässler, 1983). This suggests that the altered sensory information provided during this stage of development is not used to remodel the central circuitry underlying leg movements and coordination.

9.1.4.2 Control of reflex gain The reflexes elicited by sense organs are generally flexible. This is important in many situations because it permits a reflex to be suppressed or overridden if it is inappropriate to the behavioural context. This is particularly clear in the case of leg reflexes mediated by the femoral chordotonal organ. For instance, if all tibial movements elicited resistance reflexes as described in Section 9.1.4.1, then any attempted voluntary movement would immediately be counteracted.

In both the locust and stick insect, the gain of femoral chordotonal organ reflex loop can change markedly (Field and Burrows, 1982; Zill, 1985b; Kittmann, 1991). In an inactive stick insect, for instance, tactile stimulation can cause the gain to increase by a factor of 50 (sensitisation). Similar increases may also occur spontaneously. On the other hand, repetitive stimulation of the femoral chordotonal organ by movements of its apodeme causes a gradual decrease in gain (habituation). Interestingly, when the entire leg is moved repetitively in a similar way so that the femoro-tibial joint cyclically flexes and extends, the gain of the reflex habituates only a little (Bässler and Nothof, 1994). The difference is suggested to be caused by the action of load-sensitive campaniform sensilla located on the basal leg joints. Stimulation of the campaniform sensilla affects the gain of not only the ipsilateral femoral chordotonal organ control loop, but also the contralateral loop. It therefore acts in a similar way to a general sensitising stimulus such as tickling the animal. Kittmann (1991) suggests that the flexibility permits effective feedback, while preventing instability. That the system may be close to instability under some natural conditions has been shown by Bässler et al. (1974). The femoro-tibial control system can be induced to produce oscillations by increasing the mass of the tibia; by introducing a delay into the control loop; or by reversing the sign of the feedback (see Bässler and Nothof, 1994; Pfeiffer et al. 1993). During a sequence of such oscillations the reflex gain decreases (Bässler and Nothof, 1994), as it does for imposed movements of the femoral chordotonal organ. This supports Kittmann’s proposal that the habituation acts to prevent feedback oscillations, thus permitting the system to work close to instability. In the stick insect, a high gain is an important prerequisite for the production of catalepsy, a behaviour in which the legs remain almost motionless for long periods of time (see Bässler, 1983, 1993).
The gain of the locust femoral chordotonal organ system is lower than that of the stick insect (Ebner and Bässler, 1978), and the animal does not display catalepsy.

The neuronal elements responsible for alterations in reflex gain have been examined in both the locust (Burrows et al. 1988) and stick insect (Büschges, 1990). Populations of non-spiking interneurons exert strong influences on the motor neurons controlling the tibia. In the locust, and probably also in the stick insect, they act as summing points for both local and intersegmental inputs (see Section 9.1.2.5). Injection of current into a single non-spiking local interneuron can greatly alter the strength of a reflex mediated by the femoral chordotonal organ in both stick insects and locusts. Furthermore, in the locust, inputs onto these same interneurons from intersegmental interneurons that carry information from an anterior ganglion are similarly able to effect changes in the gain of a local reflex (Laurent and Burrows, 1989b). This latter pathway provides a way in which local reflexes can be placed into the context of the activity of other legs (Burrows, 1994). It would be valuable to now investigate (A) if the membrane potentials of these interneurons fluctuate appropriately in a behaving animal to drive the observed changes in reflex gain; and (B) if the load-sensitive campaniform sensilla mentioned in Section 9.1.4.2 make connections with non-spiking interneurons that mediate the femoral chordotonal organ reflex. This would be a simple pathway through which load information could increase the reflex gain.

Femoral chordotonal organ reflex gain is also controlled by presynaptic inhibitory mechanisms. These are discussed in Section 9.3.

9.1.4.3 Assistance reflexes

Not only the gain, but also the sign of chordotonal organ reflexes can change. This phenomenon of reflex reversal has been particularly well studied in the stick insect (Bässler, 1973, 1974, 1983). In a quiescent restrained stick insect, stretch of the femoral chordotonal organ causes a reflex extension of the tibia, whereas in an active animal, stretch generally leads to tibial flexion. Relaxation of the FeCO has the opposite reflex effects. The reflex reversal can be seen in the responses of a single motor neuron (Bässler, 1976). For instance, stretch of the FeCO in an inactive stick insect causes a depolarisation of the slow extensor motor neuron, whereas an identical stimulus given in an active animal causes a hyperpolarisation.

In inactive animals (both locust and stick insect), a given stimulus of the femoral chordotonal organ can elicit a reflex that contains both assistance and resistance components (Field and Burrows, 1982; Bässler et al. 1986). For example, slow relaxation of the femoral chordotonal organ apodeme causes SETi to be hyperpolarised, as expected for a resistance reflex. Faster relaxations, however, first depolarise and then hyperpolarise SETi. It appears that acceleration receptors of the femoral chordotonal organ are responsible for the initial depolarisation. In actively moving animals, reflexes serve to stabilise joint velocity (see Section 9.1.4.1) but superimposed on these predominant effects are smaller brief increases in velocity at the
beginning of movements in either direction, presumably corresponding to the effect mediated by acceleration receptors as described above.

In active stick insects, slow elongation of the femoral chordotonal organ (mimicking flexion of the tibia from nearly fully extended angles to nearly fully flexed angles) elicits the so-called active reaction (Bässler, 1988). The active reaction is a behaviour consisting of at least two components. First, the early part of the flexion (i.e. while the tibia is still relatively extended) elicits an assistance reflex that serves to enhance the imposed movement (as described above). This reflex is velocity dependent: at higher velocities a resistance reflex is seen instead. It thus controls the velocity of the flexion, as described by Weiland and Koch (1987). The flexor motor neurons are excited, while FETi and SETi are inhibited. Once the tibia reaches a critical flexed angle, the assistance reflex of the active reaction gives way to a resistance reflex in which FETi and SETi fire, and the flexors are inhibited. This component is thus position-dependent, and may normally contribute to the transition from stance to swing. The overall active reaction is mediated by influences from both the femoral chordotonal organ and load-sensitive campaniform sensilla. If the trochanteral campaniform sensilla are ablated, stretch of the femoral chordotonal organ apodeme is less likely to elicit an active reaction than it is in an animal with intact campaniform sensilla. The interactions between coxal sense organs and the femoral chordotonal organ are further illustrated in experiments on stick insects performing searching movements (Karg et al. 1991). It is suggested that a coxal hair plate initially signals coxal depression and induces a slow flexion of the femoro-tibial joint. The active reaction, mediated by the femoral chordotonal organ, then amplifies this movement to produce the final motor output, which consists of a combined coxal depression and tibial flexion. Active reactions are seen during forward walking, backward walking and searching movements (Nothof and Bässler, 1990). Relaxations of the femoral chordotonal organ do not cause an active reaction.

Zill and Jepson-Innes (1990) describe two reflex modes in standing locusts *Schistocerca gregaria* and *S. americana*: one is a resistance mode, in which flexor and extensor tibiae motor neurons fire appropriately to resist imposed movements of the femoral chordotonal organ (mimicking tibial movements); the other is the so-called “flexor reflex” mode, in which flexor motor neurons fire in response to both flexions and extensions of the tibia. Flexor reflexes occur only when the tibia is almost fully flexed, at which point the extensor muscle has a low mechanical advantage. Zill and Jepson-Innes (1990) suggest that they may serve to move the tibia in this situation when load-compensatory reflexes mediated by the extensor muscle would be ineffective. An alternative explanation is that they serve to prepare the animal for jumping by fully flexing the tibia.

Field and Rind (1981) describe intersegmental resistance and assistance reflexes mediated by the femoral chordotonal organ of the weta *Hemideina femorata*. One of the intersegmental effects (onto the levator trochanteris muscle of the same leg) is a resistance reflex; the others are assistance reflexes which may participate
in locomotion. They could assist leg retraction and lift the tarsal claws during protraction. For example, tibial extension (which, in the hind leg, occurs during stance phase of locomotion) activates the depressor tarsi and retractor unguis muscles, which could increase traction of the tarsal claws with the substratum during the power stroke. At extended tibial angles (as occur at the end of stance), tibial extension also activates the levator tarsi, perhaps serving to lift the claws clear of the substratum during the swing phase.

Recordings from spiking interneurons with somata at the ventral midline of the stick insect ganglion indicate that the change in reflex properties that accompanies a change in the activity state of the animal occurs before or at this neuronal level (Bässler and Büschges, 1990). In other words, the responses of some spiking local interneurons to stimulation of the femoral chordotonal organ are different in active and inactive stick insects. It is not clear, however, how (or if) these interneurons contribute to the reflex, or what neuronal pathways convey the femoral chordotonal organ information to them. In the locust, there are both direct and indirect pathways.

In both the locust *Schistocerca gregaria* and stick insect *Carausius morosus*, particular non-spiking interneurons appear to underlie both assistance and resistance reflexes (Burrows et al. 1988; Büschges, 1990). For example, interneurons of Types E4, E5 and E6 in the stick insect (Büschges, 1990) excite SETi and are depolarised by both imposed extensions and imposed flexions. It therefore appears that at least some reflex movements are the result of a summation of underlying resistance and assistance components. In addition, as for spiking interneurons, the responses of some non-spiking interneurons to movements of the femoral chordotonal organ change as the behavioural state of the animal changes (neurons E3 and I2; Bässler and Büschges, 1990).

The changes in reflex responses to femoral chordotonal organ stimulation that accompany changes in the activity state of the stick insect are already apparent at the level of both spiking and non-spiking interneurons (Bässler and Büschges, 1990). Future experiments should attempt to further define the point at which these changes take place.

9.1.4.4 *Hysteresis in the femoral chordotonal organ control loop* The responses (firing rate, membrane potential) of many neurons in the femoral chordotonal organ control loop differ for a given tibial position or movement depending on the history of movements (see Section 7.4.2.1 for a discussion of hysteresis in primary afferents).

Spiking local interneurons of the locust metathoracic ganglion that receive direct excitatory inputs from femoral chordotonal organ afferents can also show position hysteresis (Burrows, 1988), although not all do so. For instance, one extension-velocity-sensitive interneuron is initially silent at a tibial angle of 80°, but following a brief flexion and extension movement that transiently inhibits and then excites it, it begins firing tonically at approximately 15Hz when the leg is again at 80°. Burrows (1988) states that this long-lasting (e.g. 3s) hysteresis outlasts changes in the pattern of the afferent spikes but, as shown by
Matheson (1992b), most position sensitive afferents do show hysteresis over this length of time. The extracellular recording technique used by Burrows most likely failed to detect the smaller spikes of the position-sensitive afferents. Hatsopolous et al. (1995) show, using a modelling approach, that presynaptic inhibition between femoral chordotonal organ afferents (see Section 9.3) could act to reduce hysteresis in their postsynaptic targets. They claim that spiking local interneurons display less hysteresis than do the primary afferents, and invoke presynaptic inhibition to explain the difference. Currently, however, there is insufficient evidence to substantiate the claim that the extent of hysteresis differs at different levels in the control loop. Their claim is apparently based on only one recording in Burrows (1985b) which shows a spiking interneuron that has little hysteresis. In contrast, Burrows (1988) shows another spiking interneuron that has considerable hysteresis, and states that the effects seen in some spiking interneurons are similar to the marked effects seen in the non-spiking interneurons described by Siegler (1981). Thus, presynaptic inhibition between afferents can theoretically reduce hysteresis in postsynaptic neurons, but whether this actually occurs is unclear. It may be possible to test this by blocking the GABAergic presynaptic inhibition, but it will be difficult to selectively block this without markedly affecting other pathways mediated by the same transmitter.

Local nonspiking interneurons and leg motor neurons also show hysteresis. Siegler (1981) states that non-spiking interneurons invariably show hysteresis, which can be as great as 33% of the total difference in membrane potential recorded at the extremes of tibial movement. The hysteresis can last for several minutes. The SETi motor neuron shows a clear hysteresis for at least 30s. Such long-term effects have not been studied in other parts of the control system. In these experiments the entire tibia was moved, so many sense organs could have contributed to the response. Field and Burrows (1982) show that the number of spikes elicited in SETi by a small movement of the femoral chordotonal organ apodeme alone differs if the starting position is approached by a stretch or relaxation. The apodeme was set to the starting angle 30s before the test movement. The hysteresis represents approximately 35% of the maximum number of spikes recorded. Similar values are given for SETi in standing animals following a small tug on the femoral chordotonal organ apodeme (Zill and Jepson-Innes, 1990). In the stick insect (Büschges, 1990), hysteresis in non-spiking interneurons is also evident following movement of the femoral chordotonal organ apodeme alone. Although hysteresis was not studied in detail in this paper, it is possible to calculate that one interneuron (Type E3) had a hysteresis of approximately 41% of the overall change in membrane potential for movements between 50 and 110°, whereas another (Type E6) had a 20% hysteresis calculated in the same way. Note, however, that these percentages are overestimates, because the total change in membrane potential would be greater if it had been measured over the full range of tibial angles. Even so, the absolute values of hysteresis (0.3 and 1.4 mV, respectively) should contribute markedly to the integrative properties of these interneurons.
Hysteresis has typically been viewed as a coding problem that must be overcome by the CNS in order to extract meaningful information from an ambiguous signal. An alternative view has been suggested for hysteresis in the femoral chordotonal organ control loop: Siegler (1981) suggests that it may act to match the firing properties of motor neurons to the contractile properties of the muscles that they innervate. Muscles are well known to show length and tension hystereses. Zill and Jepson-Innes (1988) take this idea further, and show that the hysteresis of motor spikes following movements of the femoral chordotonal organ apodeme allows the muscle tension to fall rapidly to prestimulus levels with no overshoot. This mechanism therefore does appear to be involved in overcoming nonlinearities in the muscles.

9.1.4.5 Range fractionation in the femoral chordotonal organ control loop Both tonic and phasic afferent neurons of the locust and stick insect femoral chordotonal organs show range fractionation (Zill, 1985a; Matheson, 1992b; Hofmann et al. 1985) (see Section 7.4.2.1). Midline spiking interneurons maintain the range fractionation, and probably exaggerate it: Burrows (1988) states that no interneuron recorded in his study responded over the full range of tibial angles. In contrast, convergence of afferent input can mean that individual interneurons are excited by both directions of movement; a response not seen in any afferent. The strength of reflexes mediated by the femoral chordotonal organ differs at different tibial angles (Field and Burrows, 1982; Field and Coles, 1994). It is possible that these differences result from the activation of different afferents and, in turn, interneurons, at each tibial angle that then drive the motor neurons with more or less strength. For instance, extension movements from flexed tibial angles elicit stronger reflex responses in flexor motor neurons than do equivalent extensions that start from more extended angles. Interneurons that are excited most strongly by extension movements near full flexion may make stronger connections with flexor motor neurons than do corresponding interneurons that are activated at more extended angles. The strengths of these connections should now be tested.

In addition to angular range fractionation, there is evidence for fractionation of the range of velocities coded by the femoral chordotonal organ (Matheson, 1992b). For instance particular afferents are markedly more sensitive to low velocities of movement than are other afferents. They also begin to saturate at lower velocities. The fractionation of velocity responses appears to be more marked in midline spiking interneurons (Burrows, 1988). These interneurons may be excited by low velocities but inhibited by faster movements. This seems to represent a type of lateral inhibition to sharpen the response properties of the interneurons.

9.1.5 Interjoint and intersegmental reflexes mediated by the femoral chordotonal organ The femoral chordotonal organ mediates reflexes not only of the femoro-tibial joint, but also of other joints of the same leg (Burrows and Horridge, 1974; Field and Rind, 1981). In the New Zealand weta Hemideina femorata (Stenopelmatidae),
stimulation of the femoral chordotonal organ elicits reflexes in five muscles of the tibio-tarsal and coxo-trochanteral joints as well as the two muscles of the femoro-tibial joint (Field and Rind, 1981). The reflex in the levator trochanteris seems to be a resistance reflex, whereas those in the levator tarsus, depressor tarsus, retractor unguis and depressor trochanteris seem to be involved in locomotion, perhaps serving to enhance leg retraction and tarsal levation.

It is not as clear what reflexes the femoral chordotonal organ might elicit in adjacent legs. Ablation of the femoral chordotonal organ generally has little effect on stepping coordination (for a review, see Delcomyn, 1985). Laurent and Burrows (1989a,b) describe intersegmental neural pathways that could mediate reflexes of adjacent legs, but possible reflexes were not investigated. The mesothoracic descending interneurons that they studied receive inputs from the femoral chordotonal organ and from exteroceptors, and make output connections with ipsilateral metathoracic retractor unguis, depressor trochanteris, levator trochanteris, extensor tibiae or flexor tibiae motor neurons, or with metathoracic non-spiking interneurons. Most of the recorded effects are excitatory. Laurent and Burrows (1989a,b) did not specifically test the effects of femoral chordotonal organ stimulation on these pathways; instead they relied mainly on tactile stimulation of exteroceptors. Nevertheless, it seems reasonable to suppose that stimulation of the mesothoracic femoral chordotonal organ could lead to changes in the activity of metathoracic motor neurons through these pathways, or to presynaptic inhibition of metathoracic FeCO afferents (see Section 9.3).

9.2 PROCESSING OF INFORMATION FROM OTHER CHORDOTONAL ORGANS

9.2.1 Coxal

Two thoraco-coxal chordotonal organs (anterior and posterior joint chordotonal organs, ajCO, pjCO) and a complex system of four thoracic chordotonal organs (anterior, posterior, coxal, ventral; aCO, pCO, cCO, vCO) monitor position and movement of the mesothoracic coxa (Fig. 6b; see Section 7.4.1). The number and arrangement of homologous organs in the pro- and metathorax are somewhat different (see Section 7.4.1)

Artificial movements of the mesothoracic cCO strand of a locust throughout its antero-lateral to postero-medial range of movement (presumably stimulating cCO, aCO, pCO and vCO) elicit reflexes in the ipsilateral thoraco-coxal and coxo-trochanteral muscles. The reflexes seem to be primarily elicited by cCO, as immobilising aCO, pCO and vCO does not significantly alter the observed response. During walking, partial restraint of the cCO apodeme causes marked changed of the leg movements. The chordotonal organ system mediates both assistance and resistance reflexes in different motor neurons. As for the femoral chordotonal organ system, the gain of coxal chordotonal organ reflexes can change markedly under different circumstances, but Hustert (1982) never observed reflex reversal. The resistance reflexes occur late in protraction and retraction, and
may, therefore, act to terminate the swing and stance phases, respectively. Some contralateral coxal motor neurons are also reflexly influenced by movements of the cCO strand. In none of the above cases is it known whether the reflexes are mediated by direct or indirect neural pathways, but the lability and opposing signs of some of the effects indicate that at least some indirect pathways are present. Elongation of a coxal chordotonal organ associated with muscle 180 of a cockroach leg (mimicking coxal depression) produces resistance reflexes in coxal levator muscles, but no effects in coxal depressors (Brodfuehrer and Fourtner, 1983).

There are at least ten other sensory receptors in the thoraco-coxal joint, and it is not known how their responses interact with those of the chordotonal organs. Hustert (1982) summarises what is known of these different receptors. It is also not known in this system whether individual chordotonal organ afferents respond to only one or to both directions of strand movement. A further question of importance, although difficult to answer, is how (or whether) the complex arrangement of the chordotonal organ system in some way simplifies the neural processing that must be required to drive the equally complicated muscular arrangement.

9.2.2 Wing

The influence of sense organs on the flight pattern-generator has been the subject of considerable study, but most investigations have focused on the wing stretch receptor, a non-chordotonal afferent. For example, Wilson and Gettrup (1963) and later, Kutsch (1974), showed that destruction of wing sense organs, including both the stretch receptor and chordotonal organ, causes the wingbeat frequency of adult locusts (*Locusta migratoria*) to decrease by 40-50%. It thereafter increases over a period of several days, finally reaching 75% of the original value. The wing chordotonal organ contains approximately 21 neurons, located very near the stretch receptor (Fig. 5b). These afferents are strongly activated during flight (Pearson *et al.* 1989), but the activity is only weakly modulated in time with the flight rhythm. The receptors are also activated by low frequency sound (near 3 kHz) and to vibrations of the substratum (Möss, 1971 [in *Gryllus campestris*]; Pearson *et al.* 1989). When they are artificially stimulated at different phases of the motor output they have little effect on the flight rhythm (Pearson *et al.* 1989). Their primary function, therefore, appears to be auditory. This conclusion is supported by their central projections (see Section 8.4.2.1) and connections (Pearson *et al.* 1989). Electrical or natural sound stimulation of the receptors failed to reveal any direct connections with flight motor neurons or interneurons (e.g. interneuron 511). In contrast, auditory interneurons (e.g. 601 [=TN1, Römer and Marquart, 1984]) do receive apparently direct inputs. At least two other interneurons (607, 608) are excited by the hindwing chordotonal organs (Pearson *et al.* 1989). These interneurons are not activated during flight, but do respond to low frequency sound, even
when the tympanal nerves are cut. It is possible that interneuron 607 (with its soma in the mesothoracic ganglion) is homologous with interneuron 608, whose soma is in the metathoracic ganglion.

Two issues remain to be clearly established. First, it is not known if all the 21 wing chordotonal organ afferents respond to sound. At least 10 do so, but it remains possible that some of the others do not, and these may have some influence on the flight motor neurons. Second, it is possible that the chordotonal organ does influence the flight motor pattern, but not in a way that could be detected by Pearson et al. (1989), who analysed only the overall wingbeat frequency. For example, they suggest that it is possible that the chordotonal organ affects the relative timing of different motor neurons. Nevertheless, it appears that the hindwing chordotonal organs do not have a strong role to play in the control of flight. Instead, they appear to contribute information about low frequency sound or substratum vibrations to auditory/vibratory pathways.

Another sense organ associated with the wings of locusts is the tegula, a complex structure consisting of a knob-like hair field and a chordotonal organ (Kutsch et al. 1980). Although the role of this organ in the control of flight has been extensively described, methodological constraints mean that it has not been possible to distinguish between the responses, postsynaptic effects or central projections of the chordotonal and hair sensilla. The effects described below for manipulations of the entire tegula cannot be ascribed particularly to its chordotonal afferents or hair afferents, so the term ‘tegula afferents’ is used to cover both types, unless stated otherwise.

Forewing tegula afferents (probably both hair and chordotonal sensilla) fire shortly after the wing has passed the upper reversal point and remain active during the downstroke (Neumann, 1985). Pearson and Wolf (1988) have shown that hindwing tegulae afferents evoke EPSPs in wing elevator motor neurons and IPSPs in wing depressors, results which directly contradict earlier work by Kien and Altman (1979) which appears to be incorrect in this respect. Ablation of the hindwing tegulae delays the onset of elevator activity relative to the preceding depressor burst, so it seems that the main role of the hindwing tegulae in flight is to generate the initial depolarisation in forewing and hindwing elevator motor neurons (Pearson and Wolf, 1988). Such ablation delays the wing upstroke and causes a reduction in net lift production (Wolf, 1993). These changes in the flight system following ablation of the tegulae soon recover (Büschges and Pearson, 1991), accompanied by reorganisation of the central projections of the afferents (Büschges et al. 1992).

9.2.3 Cercus
The cerci of the cockroach *Periplaneta americana* each possess a chordotonal organ containing approximately 70 afferents with axons in nerve X (Bernard
et al. 1983). Electrical stimulation of the chordotonal organ causes an IPSP in giant interneurons GI1, GI2 and GI3, with a latency of 1.4-1.8ms. It reliably follows stimulation frequencies of up to 75Hz. Bernard et al. (1983) say that they cannot rule out the possibility that the IPSP is mediated by monosynaptic connections from the afferents onto the interneurons. This seems unlikely, however, because inhibitory mechanosensory afferents have so far not been described in insects. The cercal chordotonal organ responds to movements of the cercus (Callec, 1972), but the specific response properties of the afferents or their overall reflex effects on cercal motor neurons (if any) are not known. Bernard (1987) shows that the function of the inhibitory PSP in the giant interneurons is to prevent an inappropriate escape response being elicited by the wind sensitive cercal hairs during (voluntary) movements of the appendage. Wind sensitive hair afferents of the cercus presynaptically inhibit each other through an indirect pathway (Blagburn and Sattelle, 1987) and receive inhibitory presynaptic inputs from another receptor (or receptors) of the cercus (Boyan, 1988), but it is not known if the source of the latter inputs is the chordotonal organ (see Section 9.3.4).

9.2.4 Convergence of information from different chordotonal organs

There is little direct information about the convergence of afferent information from different chordotonal organs onto common central target neurons. Such interactions might be expected, because they could underlie the coordination of leg joints or legs. Several observations suggest possible pathways by which such interactions may occur. Already mentioned (Section 9.1.2.4) are descending interneurons of the locust mesothoracic ganglion that carry information from mesothoracic leg chordotonal organ afferents (Laurent, 1986) to the metathoracic ganglion, where they influence local non-spiking interneurons that mediate metathoracic femoral chordotonal organ reflexes (Laurent and Burrows, 1989a,b). Some of the descending interneurons respond to movements of both the tibia relative to the femur, and the tarsus relative to the tibia. This suggests that receptors at these two joints converge here. Receptors from the tarsal chordotonal organ at least provide direct inputs (Laurent, 1987a). It would now be of great interest to determine if femoral chordotonal organ afferents also directly excite these interneurons. The local non-spiking interneurons that are the metathoracic output targets of the descending interneurons represent a further important summing point for chordotonal organ information from adjacent segments of the body.

A second observation that suggests an (indirect) interaction of chordotonal organ afferents is that imposed movements of the mesothoracic tibia elicit presynaptic inhibitory inputs in metathoracic femoral chordotonal organ afferent central terminals (T. Matheson, unpublished observation). The interneurons that mediate this effect are unknown, but are probably not the same as those mentioned above (Laurent and Burrows, 1989a).
There is presently no evidence that afferents from the tibio-tarsal and femoro-tibial chordotonal organs converge on spiking local interneurons, despite many published recordings of the receptive fields of these interneurons (e.g. Burrows and Siegler, 1984; Nagayama, 1989).

9.3 PRESYNAPTIC INHIBITION

Many studies of both vertebrates and invertebrates have now shown that presynaptic inhibition of primary afferent neurons (and other neurons) is an important, perhaps universal, aspect of signal processing (for reviews, see Atwood and Tse, 1993; Watson, 1992). Presynaptic inhibition is a mechanism by which one neuron influences the efficacy of the output synapses of another neuron, by acting on the synaptic release mechanism. In many cases the effect is mediated by gamma aminobutyric acid (GABA).

9.3.1 Anatomical and ultrastructural evidence

Electron microscopic examination of locust ganglia reveals that afferents from the femoral chordotonal organ receive many input synapses on their terminals within the neuropil (Watson et al., 1993). Individual afferents were filled intracellularly with horseradish peroxide, and the tissue processed for GABA immunoreactivity. The input synapses onto the femoral chordotonal organ afferents were made onto terminal branches that contained vesicles, and were commonly associated with output synapses. 78% of examined input synapses were immunoreactive to GABA. The remainder, which were interspersed amongst the GABA immunopositive synapses, were not further characterised. In other insect sensory systems, however, some presynaptic inputs are glutamatergic (Watson and Pflüger, 1984), so the same is possible here. A single femoral chordotonal organ afferent can receive inputs from both GABA immunopositive and GABA immunonegative presynaptic elements. In the crayfish, presynaptic inhibition of chordotonal organ afferents is mediated by both GABA and histamine (El Manira and Clarac, 1994), a transmitter that is also present in the insect visual system. It would be valuable to test for this substance in neurons presynaptic to locust femoral chordotonal organ afferents.

The close apposition of input and output synapses suggests that the presynaptic regulation of information flow is fairly specific. Nevertheless, the presynaptic elements (which have not yet been identified) also make output synapses onto other afferent neurons that appear not to be chordotonal afferents.

9.3.2 Physiological evidence

Physiological aspects of the presynaptic inhibition of locust femoral chordotonal organ afferents have been studied in detail by Burrows and Laurent (1993), Burrows

This work has taken two complementary approaches: (1) recording from the central branches of individual afferents during imposed and voluntary leg movements; and (2) recording the afferents’ responses to applied neurotransmitters and drugs.

These experiments have concentrated on an aspect of presynaptic inhibition that has not previously been documented; namely, indirect interactions between the afferents from a single chordotonal organ. Earlier descriptions of presynaptic inhibition in other systems have concentrated on inputs from other sense organs or from central pattern generators.

The main features of presynaptic inhibition of locust femoral chordotonal organ afferents are these. First, there is no evidence that the afferents interact directly with each other, although this possibility is not entirely ruled out. Second, the afferents receive inputs from at least three sources: (1) indirectly from other FeCO afferents; (2) indirectly from other receptors of the same or different legs (T. Matheson, unpublished observation; see Wolf and Burrows, 1995); and (3) from central pattern generators (Wolf and Burrows, 1995). The interneurons that mediate these different effects may form a single population, or may be separate populations. Third, the inputs are depolarising inhibitory potentials, mediated by GABA, which act to shunt the afferent spike and reduce transmitter release. This reduces the strength of the connections made by the afferents onto postsynaptic leg motor neurons. Fourth, the afferents interact indirectly in specific combinations, so that spiking activity of any given afferent is always superimposed on a barrage of inhibitory inputs. The most effective stimulus for a given afferent is also the stimulus that generates the greatest presynaptic inhibition.

9.3.3 Functions of presynaptic inhibition

The evidence reviewed in the previous section suggests that the presynaptic inhibition forms an automatic gain control mechanism, in which the pooled and weighted effects of specific groups of FeCO afferents act indirectly on other FeCO afferents to attenuate the strength of their reflex effects. The overall effect of this would be to reduce the strength of the reflexes mediated by the femoral chordotonal organ, perhaps preventing the postsynaptic motor and interneurons becoming saturated with the convergence of many afferents all firing at high frequencies.

An additional function has been proposed by Hatsopolous et al. (1995). They show that, in the same system, presynaptic inhibition could theoretically act to remove a large proportion of the hysteresis that is observed in the primary afferent responses. This has not been confirmed experimentally, and there is no good evidence that hysteresis is less marked within the CNS. Their modelling approach also confirmed the earlier suggestion that an automatic gain control system could prevent saturation of a postsynaptic target.
9.3.4 Other aspects of presynaptic inhibition

A chordotonal organ at the base of each cercus of the locust has been indirectly implicated in the presynaptic modulation of sensory information from cercal wind-sensitive hairs (Boyan, 1988). The identity of the sensory neuron responsible for the observed physiological effects has not clearly been established, however, because its peripheral soma has not been located. The single central fill of the afferent (Boyan, 1988) is not sufficient to determine if its projections match those reported for the cockroach cercal CO. The branches of the afferent stained in the locust are much more restricted in distribution than those illustrated for the entire cercal CO (Füller et al., 1981). This observation does not rule out the possibility that the afferent is from the CO, because it is likely that some receptors in the CO branch only in restricted areas (as already shown for the locust FeCO (Matheson, 1992a)).

Presynaptic inhibition of wind-sensitive hairs (by the presumed cercal CO) is associated with a membrane depolarisation and reduction in spike amplitude, similar to that seen in FeCO afferents (see Section 9.3.2), and is probably also mediated by interneuronal pathways (Boyan, 1988). Its function is apparently to prevent self-induced stimulation of the wind-sensitive afferents from eliciting inappropriate escape responses (see Bernard, 1987 for evidence in the cockroach). Interestingly, the afferent responsible for the presynaptic inhibition also synapses directly onto one of the giant interneurons involved in escape (which also receives input from the same filiform hairs), which it inhibits by increasing the membrane conductance.

9.4 Neuromodulation

Neuromodulation of chordotonal organ responses has been studied in most detail in the stick insect FeCO (Büschges et al., 1993; Ramirez et al., 1993). The biogenic amine octopamine causes behavioural changes that are similar to those elicited by general tactile stimulation (i.e. arousal). At the same time, leg reflexes mediated by the FeCO are markedly altered. Both central and peripheral effects appear to contribute to these changes.

9.4.1 Central modulation

Bath application of octopamine (5×10⁻³ mol l⁻¹) onto the mesothoracic ganglion of the stick insect causes two sequential changes in FeCO reflexes. First, the animal becomes more active, and FeCO reflexes become more variable. This resembles the situation in an animal that has been aroused by tactile stimulation (Büschges et al., 1993). Second, the animal becomes inactive, and FeCO resistance reflexes in response to passive tibial movements are completely suppressed. During this phase the animal can still generate active
movements, indicating that octopamine acts particularly on the circuitry that mediates resistance reflexes, and not on that which generates active movements. In this case, octopamine probably acts primarily on interneurons, because the general excitability of the postsynaptic motor neurons was not markedly altered.

9.4.2 Peripheral modulation
In addition to its effects on the central pathways mediating FeCO reflexes, octopamine also has direct affects on the primary afferent neurons (Ramirez et al. 1993). Tonic (position sensitive) activity is enhanced at all tibial angles by concentrations as low as $5 \times 10^{-7}$ mol l$^{-1}$. This is also reflected in recordings of individual receptors. Interestingly, velocity sensitivity is unaffected by octopamine, even in neurons that respond to both position and velocity, and whose position sensitivity is altered. The system desensitises within 15 min, with subsequent applications of octopamine being ineffective.

9.4.3 Effects of octopamine in the locust
In the locust Schistocerca gregaria, octopamine has similar effects to those reviewed in the previous section (Matheson, 1994, 1997). These experiments have so far added three further observations. First, the octopamine agonist synephrine mimics the effects, supporting the suggestion that they are mediated by a specific octopamine receptor. Second, in contrast to the situation in the stick insect, the effect on the overall firing rate of position sensitive afferents is greatest at fully flexed angles and least at fully extended angles. This means that instead of simply shifting the entire firing frequency versus angle curve to a higher frequency, octopamine extends the range of frequencies used to code for position. This should increase the sensitivity of the system to position stimuli. Third, and most interestingly, peripheral application of octopamine to the FeCO increases not only the tonic firing rate, but also tonic presynaptic inputs to the afferents. This raises at least two possibilities: either (a) the increased afferent firing is simply down-regulated by the presynaptic inhibition so that there is no overall change in input to the CNS, or (b) the inhibition does not match exactly the increased firing in particular afferents, so that the balance of afferent input to the CNS alters (e.g. in favour of tonic afferents). It may be possible to test these hypotheses by assessing the reflex responses of motor neurons during application of octopamine to the chordotonal organ of the locust.

9.4.4 Effects of other substances on chordotonal organs
The imidazoline phentolamine, a vertebrate alpha-1 adrenergic antagonist, is known to antagonise invertebrate octopamine receptors and to have local
anaesthetic actions through block of sodium channels. Bath application of 2x10^{-4} - 3x10^{-4} mol l^{-1} phentolamine inactivates the wing CO, the FeCO, auditory afferents and the tegula CO of Locusta migratoria and Periplaneta americana (Ramirez and Pearson, 1990). Phentolamine appears to exert this effect not through octopamine receptors, but by direct action on spike generation (i.e. block of sodium channels). The local anaesthetics tolazoline and metoclopramide also selectively inactivate insect mechanoreceptors, but yohimbine and chlorpromazine do not.

The pyrethroid insecticides deltamethrin, tetramethrin, transpermethrin, fenvalerate and fluvalinate act on the locust (Locusta migratoria) metathoracic femoral chordotonal organ at concentrations near 10^{-7} mol l^{-1} (Clements and May, 1977; Theophilidis et al. 1993). At low concentrations (1.36x10^{-8} - 2.72x10^{-8} mol l^{-1}) deltamethrin causes a transient increase of firing rate to about 120% of normal for 2-3 min, and then depresses the rate to 5-10% below normal. At higher concentrations (e.g. 4.06x10^{-8} - 8.12x10^{-7} mol l^{-1}) deltamethrin has a similar pattern of effect, but the firing rate is reduced to zero within 10 min. At yet higher concentrations (e.g. 8.12x10^{-6} mol l^{-1}) there is no transient increase, and the rate falls to zero within 6 s. The drug appears to exert its effects initially on the organ itself and then on the sensory axons (Theophilidis et al. 1993). Fluvalinate reduces the firing rate to zero within 5 min at concentrations above 3.33x10^{-7} mol l^{-1}. At this concentration the drug acts primarily on the organ, and only at higher concentrations (e.g. 1.65x10^{-6} mol l^{-1}) does it act on the sensory axons. The insect femoral chordotonal organ appears to be particularly sensitive to these insecticides.

10. Development of chordotonal organs

Study of the developing insect nervous system helps to establish general principles of nervous system development that would be difficult or impossible to study in humans and other vertebrates. Boyan and Ball (1993) argued that molecular, genetic and physiological relationships are conservatively retained in the evolution of invertebrate and vertebrate organisms, including humans. Thus chordotonal organs of Drosophila and the grasshopper are useful tools for the study of genetic control of development in sensory systems (Boyan and Ball, 1993; Jan and Jan, 1993). Chordotonal organs have been studied by developmental biologists in order to understand the embryonic lineage, morphogenesis and pathway formation of insect sense organs. Neurophysiologists have studied the postembryonic development of chordotonal organs to understand how sensory systems establish and retain physiological sensitivity through successive molts during maturation. Apart from two early studies (Slifer, 1935; Jägers-Röhr, 1968), all work on chordotonal organ development is recent and has not been reviewed specifically. Broader reviews include development of arthropod sensory
systems (Bate, 1978), and *Drosophila* peripheral nervous system development and genetics (Jan and Jan, 1993).

The life histories of insects reflect their differing pattern of development. In hemimetabolous insects (e.g. Orthoptera and Blattaria) the embryonic stage lasts 2-3 weeks after which a *nymph* hatches, looking very much like a miniature adult without wings. A postembryonic phase of several weeks is required for completion of development as the insect passes through successive *instars* (moulting stages) to an adult. The degree of embryonic development is expressed as a percentage of elapsed time from egg-laying to hatching, whereas the postembryonic phase is expressed as instar number (variable between species, but rarely exceeding 10). In holometabolous insects (Diptera, Lepidoptera) the life history includes four stages: egg, larva, pupa and adult. The embryonic phase within the *egg* leads to development of a *larva* upon hatching. The larva (e.g. caterpillar, maggot) forms a *pupa* which is a quiescent stage during which metamorphosis occurs and leads to emergence of the adult. The embryonic phase is expressed as a *stage* (characterised morphologically by Campos-Ortega and Hartenstein, 1985) or as hours of development at 23-25°C (Jan and Jan, 1993).

10.1 TAXONOMIC OVERVIEW

The larval pleural chordotonal organs of the dipteran *Drosophila* have played a major role in studies that have established cell lineage, morphogenesis, differentiation, genetic control and pathway establishment of insect sensory systems. Blowfly leg chordotonal organs also are used for developmental studies of morphogenesis and pathfinding (Jan and Jan, 1993; Lakes and Pollack, 1990).

The large size of the developing embryo in the orthopteran locust *Schistocerca americana* (Acrididae) has aided studies on morphogenetic and molecular mechanisms of pathfinding in the leg FeCO and SGO, the wing-hinge chordotonal organ and the abdominal auditory organ (Müller’s organ) (Heathcote, 1981; Keshishian and Bentley, 1983a-c; Meier and Reichert, 1990). Evidence for intraspecific and interspecific homologies amongst chordotonal organs has come from developmental studies of *Schistocerca* (Meier *et al.* 1991). Additional orthopteran studies have focused on the development and associated physiology of auditory tympanal organs in crickets (Ball and Young, 1974) and bush crickets (Rössler, 1992a,b).

In other insect orders, an early histological study of the femoral chordotonal organ in the stick insect (Phasmatodea) elucidated cell lineage and morphogenesis (Jägers-Röhr, 1968). Scolopidial cell lineage and embryonic cell differentiation of the antennal chordotonal organ of the cockroach (Blattaria) was established in an ultrastructural study (Blöchl and Selzer, 1988). Other ultrastructural work examined postembryonic development and degeneration of the apical sensory organ (Lee *et al.* 1988) in butterflies and moths (Lepidoptera), and the
10.2 SCOLPIDIUM CELL LINEAGE

Early studies established that chordotonal organs originate from epidermis, and that axogenesis occurs from the periphery into the central nervous system (Slifer, 1935; Jägers-Röhr, 1968). It was also proposed, based on light microscopy, that the cells of a scolopidium are related by lineage. For the stick insect *Carausius morosus* a single epidermal cell (sensory mother cell, SMC) appears to give rise to four cells of the final scolopidium through three mitotic divisions (Jägers-Röhr, 1968; see also review by Bate, 1978). New work shows that the pattern also occurs in mononematic scolopidia with single neurons in *Drosophila* (Fig. 30a) (Bodmer et al. 1989), but this has not been verified in other insect orders. The SMC divides to yield an “accessory cell” (ligament cell in *Drosophila* larval pleural chordotonal organs) plus a secondary cell. The latter divides to yield the cap cell plus another secondary cell. A final division yields the neuron and scolopale cell (Fig. 30a). Recent work on *Drosophila* lch5 scolopidial clones (for terminology, refer to Fig. 32), using *LacZ* reporter gene and BrdU labeling, indicates that the positions of the attachment and accessory cells in the lineage of Fig. 30a might be reversed (Brewster and Bodmer, 1995). In the proposed model, the attachment cell and ectodermal cell result from the division of one SMC daughter cell, whereas the accessory cell, scolopale cell and neurone result from two divisions of the other SMC daughter cell. The above techniques also revealed that two other chordotonal precursors (vchA and vchB, see Fig. 32) produce a lineage that yields a type II multiterminal receptor neuron and scolopidium. The lineage appears to be the same as that for lch5, except that the neuron undergoes another division to produce the scolopidial neuron and the multiterminal neuron.

For mononematic scolopidia containing two neurons (e.g. the cockroach antennal connective chordotonal organ), a different pattern is found (Fig. 30b) through positive identification of cells in the lineage using electron microscopy (Blöchl and Selzer, 1988). Here six mitotic divisions yield five cells in the scolopidium plus two sensory-neuron-like cells which degenerated during scolopidial formation and were thought not to function as neurons. Thus, in the few cases studied, two orders (Phasmatodea and Diptera) have the same pattern for mononematic scolopidia with single neurons and a third order (Blattaria) shows a unique pattern for doubly-innervated scolopidia. In all cases, however, a single lineage is derived from one epidermal precursor cell. More studies are needed to ascertain the generality of these patterns. Nothing is known of the lineage pattern for amphinematic scolopidia.

The pattern of yielding an accessory cell from the first mitotic division is consistent for chordotonal organs. This differs from the mitotic pattern found for cuticular exterosensilla in *Drosophila*, where the daughter cells from the
Fig. 30. Cell lineage patterns for chordotonal and cuticular sensilla. Each sensillum is derived from a single precursor cell, the sensory mother cell. Definitive cells of the sensillum are shown hatched. a. *Drosophila* pleural chordotonal organ, with singly-innervated scolopidium. The sensory mother cell undergoes sequential divisions to produce four cells of the scolopidium. The accessory cell forms a proximal anchoring ligament for the neurone. b. More complex chordotonal cell lineage observed for the connective chordotonal organ in a cockroach antenna (two neurons per scolopidium). Compare with Fig. 31. An accessory cell is still formed from the first division, but it forms a support cell for the distally placed attachment cell ligament. The first two sensory neurons (1, 2) degenerate after the definitive neurons (3, 4) are formed. c. Lineage for a cuticular sensillum. Unlike chordotonal organs, the two daughter cells (2, 3) do not form definitive cells of the sensillum, but each divides to produce the definitive cells. The neuron precursor may divide more than once for multiply-innervated cuticular sensilla. a,c, modified from Bodmer et al. (1989), with permission; b, modified from Blöchl and Selzer (1988), with permission.
first division of the SMC both divide before yielding final cells of the sense organ (Fig. 30c). The possible evolutionary homologies of these cells are discussed in Section 12.5.

10.3 SCOLPIDIUM CELL DIFFERENTIATION

A clear understanding of cell differentiation in the developing scolopidium (Fig. 31), and unequivocal proof that the lineage of scolopidial cells is uniclonal, came from an ultrastructural study of the cockroach antennal chordotonal organ (Blöchl and Selzer, 1988).

In the two-cell stage of scolopidial development, the accessory cell resulting from the first SMC division envelops the other daughter cell and both are elongated. The distal ends bear many microvilli (Fig. 31a).

![Fig. 31. Schematic reconstruction of different stages of scolopidial development in a cockroach antennal connective chordotonal organ. Compare with Fig. 30b. a. 2-cell stage. b. 3-cell stage. c. 3-cell stage changes into 4-cell stage by mitosis of cell 3. d. 5-cell stage where mitosis of cell 2 leads to the 6-cell stage. Accessory cell (acc) begins to surround attachment cell (ac). e. 7-cell stage with four neurons and scolopale cell (sc) formed. f. Early second 5-cell stage after degeneration of neurons 1 and 2. Terminal filum formed. g. Advanced second 5-cell stage. Peripheral cell processes and terminal filum elongate; sensillum descends toward antennal lumen. Glial cells (g) and neuronal ciliary apparatus form. h. Final second 5-cell stage. Terminal filum shortens to yield mononematic scolopidium. Neuronal dendrites elongate and somata are displaced into antennal lumen. Stippling allows tracing of envelope cell development. From Blöchl and Selzer (1988), with permission.](image-url)
By the four and five-cell stage, the apical ends have retracted and formed a receptor lymph space lined with microvilli (Fig. 31c,d), reminiscent of that seen in fully formed cuticular sensilla. Spot desmosomes (found distally) and septate junctions (found proximally) join these cells. The two neurons which later degenerate (s1 and s2 in Fig. 31d,e) form elongated distal processes (presumptive dendrites) packed with microtubules, as well as proximally extending processes.

By the seven-cell stage (Fig. 31e) four neurons are formed, and their distal processes become enveloped concentrically by the presumptive scolopale, attachment and accessory cells. The first two neurons now begin to degenerate, leaving the definitive neurons s3 and s4 at the second five-cell stage (Fig. 31f). Cilia begin to form and elongate in the distal processes of the latter, the receptor lymph space sinks and closes off apically and depositions of cap material are seen amongst the microvilli of the scolopale cell. As the scolopale cell withdraws proximally and shortens, it leaves behind a “terminal filum” of dense material (Fig. 31f,g). This expands distally and begins to form the cap, apparently through secretion from the still-interdigitating microvilli. The beginnings of scolopales are seen within the scolopale cell. The cilia are formed by this time and insert into the forming cap, but they still lack ciliary roots. Their internal cytoskeleton (axoneme) is fully-developed: the A-tubules have dense cores and bear lateral arms (see Section 4.3.3.8.1).

During the final phase of the seven-cell stage the two neuronal dendrites elongate markedly while the accessory and attachment cells achieve their final distal positions and form the tapered strand connecting to the cuticle (Fig. 31g, h). The attachment cell (cap cell) develops densely packed regions of microtubules, but the accessory cell shows no further differentiation and appears to be the least specialised of the scolopidial cells. Glial cells arrive and enwrap the neurons and developing axons. Ciliary roots now arise from the distal basal body and pass around the proximal basal body as separate branches before converging proximally into roots which extend down the dendrites (Fig. 31g). As the neurons continue to elongate, the ciliary roots grow proximally to the level of the axon origin. In this final phase (Fig. 31h) the cilium now forms its distal dilation by swelling to twice its diameter just below the cap. The terminal filum regresses into the cap, which itself enlarges and becomes multiply folded and dense. The scolopale rods become thicker and distally they are incorporated into the cap. The embryonic differentiation of the scolopidium is now completed.

10.4 MORPHOGENESIS OF CHORDOTONAL ORGANS

The different life histories of insects lead to complexities in the developmental patterns of chordotonal organs. Metamorphosis in the holometabolous insects causes a major reorganisation of sensory structures such as cuticular sense organs (Hartenstein and Posakony, 1989), but less is known of the fate of larval
chordotonal organs. Some groups of hemimetabolous insects show completion of chordotonal organ morphogenesis within the embryonic stage, whereas other groups within the same order, show continued postembryonic morphogenesis through various instars. In some species major changes occur in the moult from the last instar to the adult.

Regardless of the time at which chordotonal organs develop, the process invariably involves the determination of sense organ precursor cells from the hypodermal cell layer, and commitment of these cells as neuronal precursors through positional information. (Many papers use the term epidermis incorrectly for insect development; hypodermis correctly refers to the sub-cuticular epithelial layer of tissue). These divide and delaminate from the parent layer early in embryogenesis (Jan and Jan, 1993) and are recognisable histologically by the presence of lighter-staining nuclei in tissue prepared using iron hematoxylin (Slifer, 1935).

10.4.1 Hemimetabolous development.

10.4.1.1 Wholly embryonic development In the hemimetabolous insects, chordotonal organ development is best known in the Orthoptera. In the bush crickets (Tettigoniidae) and grasshoppers (Acrididae), leg chordotonal organs and the abdominal tympanal organ form their full complement of scolopidia by the time of hatching. This wholly embryonic development differs from that observed in the crickets (Gryllidae) and in the cockroaches (Blattaria), in which there is postembryonic chordotonal organ development (see Section 10.4.1.2).

The first sign of chordotonal organ development occurs at around 35-40% embryogenesis, ranging from 35-42% for the acridid FeCO (Keshishian and Bentley, 1983b; Slifer, 1935), 40% for the tettigoniid tibial tympanal organ and acridid abdominal tympanal organ (Meier and Reichert, 1990), to 45% for the acridid wing-hinge chordotonal organ (Heathcote, 1981). The site of differentiation is marked by an invagination in the epithelium and proliferation of a compact cluster of tapered cells with their apical ends pointing into the centre of the depression. At around 45% development, the cells (which are now differentiating in the manner reviewed above) begin to actively change orientation and/or migrate into their final positions. In the tettigoniid crista acustica (see Section 3.5.4.3) this orderly morphogenetic progression may form the basis for the tonotopic organisation of central projections of crista acustica axons in the adult CNS (Meier and Reichert, 1990). For example, three or four differentiating neurons begin to segregate out from the cell mass (which represents the future complex tibial organ) and re-orient their dendrites distally (Fig. 32a). By 50% development, 13 cells have formed a linear array with dendrites pointing distally. Examples of cell migration are seen in the acridid abdominal tympanal organ and wing-hinge chordotonal organ. In the former, a quadrant of the
differentiating mass moves antero-proximal, detaches from the epithelium and assumes the configuration seen in the adult organ before the axons begin sprouting (Fig. 32c) (Meier and Reichert, 1990). In the latter, the first two differentiating neurons of the organ migrate 150 µm from the periphery to their final positions as their growing axons trail behind to establish the chordotonal nerve (Heathcote, 1981).

By 45-50% development, axogenesis begins in chordotonal organs. In limb buds, usually the main leg nerves have already been well established by this point, or at least pioneer cells have established pathways that will develop into leg nerves (see Section 10.5). Growing chordotonal organ axons have growth cones, with filopodia, which orient toward established nerve pathways, join and grow along the pathways, and fasciculate into axon bundles within the developing nerve (Keshishian and Bentley, 1983b; Meier and Reichert, 1990; Meier et al. 1991; Lakes-Harlan and Pollack, 1993). There may be dye-coupling (revealed using the fluorescent dye Lucifer Yellow) between centrally-growing chordotonal axons (Heathcote, 1981) or between the sensory axons and the pioneer cells in some species (Lakes-Harlan and Pollack, 1993), but not others (Keshishian and Bentley, 1983b). Specific patterns of dye-coupling between scolopidial neurons, accessory cells and pioneer cells occur with apparently well-regulated timing during late stages of morphogenesis (see especially Lakes-Harlan and Pollack, 1993), but the significance of this is unknown.

In the segmentally repeated chordotonal organs of the thorax and abdomen, a common structural plan is seen at the 50-55% stage. The developing scolopidia are organised into three groups: the dorsal, lateral and ventral clusters. The axons of the dorsal and lateral clusters join the intersegmental nerve of each segment, whereas those of the ventral cluster join the anterior fascicle of the segmental nerve (Meier et al. 1991). The remarkable similarity of developmental structure in these somatic chordotonal organs has provided evidence for serial homology of what become morphologically diverse chordotonal organs in the adult (see Section 12). The dorsal cluster gives rise to the multipolar wing-hinge stretch receptor (Heathcote, 1981) in the pterothoracic (wing-bearing) segments and possibly intersegmental stretch receptors in the abdominal segments, whereas the ventral cluster produces the sternal chordotonal organ and an uncharacterised ventral sense organ (Hustert, 1974; Meier et al. 1991). The lateral cluster gives rise to the wing-hinge chordotonal organ (adjacent to the multipolar wing-hinge stretch receptor neuron) in the pterothoracic segments, the auditory organ and associated tympanal cuticular ear in the first abdominal segment, and the pleural chordotonal organs in each of the remaining abdominal segments.

After 50% development, tettigoniid and acridid chordotonal organs have usually attained the morphology found in the adult. Most authors do not describe the remaining changes in morphology, although Slifer (1935) noted that the final stages involve enlargement of the organ (FeCO), and development of a ligament which fuses to a cuticular core (apodeme) extending from
Fig. 32. Embryonic development of chordotonal organs. 

**a.** Morphogenesis of the complex tibial organ (arrows) and crista acustica (solid somata) in the bushcricket prothoracic leg at 40% and 50% stages. The crista acustica cells migrate out from the lateral edge of the complex tibial organ (40%), which itself was derived from an ectodermal invagination at the site of the arrow. At 50% development the crista cells form a linear array as they migrate out; the orderly temporal sequence of this process may underlie the tonotopic central projection of crista acustica neurons.

**b.** Morphogenesis of peripheral nerves, including those innervating chordotonal organs, in the locust embryonic metathoracic limb bud at the 40% stage. Pioneer neurons have already established nerve 5B1 (N5b1) which contains axons from the femoral chordotonal organ (FeCO) and subgenual organ. Nerve 5b2 pathway is being established by the two tarsal pioneers (Ta) and one of the tibial pioneers (Ti). Efferent axonal outgrowth (e) from the CNS is seen in nerve 3b (N3b) and nerve 5b2 (N5b2).

**c.** Morphogenesis of the auditory organ (Müller's organ) of the locust first abdominal segment (lateral view of segment, anterior to left). At 40% development an epithelial invagination (arrows) marks the appearance of the auditory organ posterior to the spiracle, near the segment border (arrowheads). The intersegmental nerve has been pioneered already by axons from the dorsal body-wall cells and from efferent neurons. At 45% development, antero-ventral cells migrate towards the intersegmental nerve. The stretch receptor homologue separates from the dorsal body wall cluster. At 50% development the auditory organ cells initiate axogenesis and join the intersegmental nerve (arrow), which becomes the tympanal nerve in the adult. Scale for all:
50µm. d. Peripheral nervous system of Drosophila embryo shown as camera lucida tracings of stained preparations (left) and

the distal side of the femoro-tibial joint. Increases in the amount of cytoplasm within scolopale cells, attachment cells, glial cells and accessory cells which surround the scolopidia, apparently account for at least some of the enlargement of the organ.

Two significant conclusions result from the above studies. First, migrating cells must be able to read positional information in the antero-posterior axis, and undergo active motility rather than passive displacement due to body growth. This arises from the observation that individual cells and cell groups migrate simultaneously in different directions along the body axis during chordotonal organ embryogenesis. Second, because cells migrate as groups rather than as individuals, cell adhesion molecules must be specifically

**Fig. 32 caption continued**

diagrammatic organisation (right) for a thoracic and an abdominal segment. Chordotonal organ neurons are filled in black. The sensory cells are organised into patterned clusters, named by Ghysen et al. (1986). In this scheme the first letter of the name indicates the cluster’s position: d, dorsal; l, lateral; v,v', ventral - of which the v' cluster is more dorsal. Subsequent letters in the name indicate sensillum type: es, external (cuticular) sensilla; ch, chordotonal sensilla. A numeral at the end of a group name indicates the number of sensilla it contains. Symbols: heavy lines, axons; thin lines, dendrites reaching the embryo surface either singly (open circles) or in groups (filled circles); triangles, chordotonal scolopidia; dotted areas, positions of cell body clusters. a and c, modified from Meier and Reichert (1990), with permission; b, modified from Keshishian and Bentley (1983b), with permission; d, modified from Ghysen et al. (1986), with permission.
expressed in a localised cluster to facilitate its movement (confirmed by the demonstration of Fasciculin I expression in developing auditory and pleural chordotonal organs of the grasshopper, Sections 11.2, 11.3.2) (Meier and Reichert 1990).

10.4.1.2 Postembryonic development An exception to the above differentiation sequence occurs in the development of the complex tibial organ (see Section 3.5.4) in the prothoracic leg of crickets (Gryllidae). The various chordotonal organs of this complex differentiate asynchronously: the SGO differentiates embryonically and at the time of hatching contains almost half the final number of scolopidia, which is reached in the fourth instar. The tympanal organ does not differentiate until the third instar, at which time its first scolopales are visible with light microscopy. Subsequent scolopidia are added right through the postembryonic development of the cricket (10 instars) in stepwise order. First the scolopidia of the “proximal group A” differentiate, then in the fifth instar the “proximal main group” scolopidia appear, and finally in the sixth instar the “distal group” differentiate. By the seventh instar the adult number of scolopidia is reached (Ball and Young, 1974).

During morphogenesis in the cricket complex tibial organ, there is sequential enlargement of all the scolopidial cells, especially the large accessory cells which anchor the scolopidia to the internal cuticular wall. The scolopales and scolopale cells are very thin until the adult stage. The dorsal insertion of the tympanal organ does not develop until the fifth instar, after which a tent-like covering membrane forms over the entire organ. The two tracheae of the prothoracic leg are simple parallel tubes in the first seven instars. In the seventh instar they become joined by an interconnection, and commence modification into acoustic chambers. By the ninth instar the tracheae have enlarged disproportionately and they occupy the entire width of the tibia in the region of the tympanal organ. In the final two mouls, the tracheal chambers contact the tympanal organ and lastly, form intimate contact with the thin cuticular tympanal membranes, which only appear at the adult moult.

Considerable postembryonic differentiation also occurs in the cockroach (Blattaria). In the antennal connective chordotonal organ, the embryo forms about 40 scolopidia by 83% development, and another 10 scolopidia are added during postembryonic instars (Blöchl and Selzer, 1988).

10.4.2 Holometabolous development

Holometabolous insects, such as flies (Diptera) and butterflies (Lepidoptera), undergo metamorphosis in the pupal stage, between the larval crawling stage and the adult. Prior to pupation, fully developed sets of chordotonal organs are formed, although their functionality has not been demonstrated. During
metamorphosis most of these larval neurons degenerate (Sprey, 1971; Kutsch and Bentley, 1987; Lakes and Pollack, 1990). Adult sensilla are born in the late third instar larva and appear to have their full complement of scolopidia by the mid-pupal stage. As seen in embryogenesis, chordotonal organs, particularly the FeCO, and multiply-innervated cuticular sensilla form before singly-innervated cuticular sensilla (Lakes and Pollack, 1990; Jan and Jan, 1993). Adult chordotonal organs take much longer to develop than embryonic chordotonal organs.

10.4.2.1 Embryonic morphogenesis Embryonic chordotonal organ development is described for Drosophila and is based upon labeling with anti-HRP and 21A4 monoclonal antibodies (Ghysen et al. 1986). Differentiation commences at stage 10 (5h into embryogenesis) when SMCs begin to express sense-organ-specific gene products, and by stage 11-12 (6-9h) scolopidial cells begin to differentiate (cell lineage shown in Fig. 30a). The process is similar to that for hemimetabolous insects (see Section 10.4.1) and was described ultrastructurally by Hartenstein (1988).

The segmentally repeated groups of larval sense organs appear in a dorsal-to-ventral sequence, and the groups maintain a fixed spatial relationship during development (Jan and Jan, 1993). Within each larval segment, the sensory cells are arranged into four recognisable clusters; minor variations between segments yield five different patterns along the thoracic and abdominal segments. The four clusters characteristic of each segment include two ventral (v and v'), one lateral (l) and one dorsal (d) cluster (Fig. 32d), in which are found both scolopidia (ch) and external cuticular sensilla (es). The numbers of scolopidia differ in the thoracic and abdominal clusters, but in both, the scolopidia eventually lie alongside each other in a row (dch3, lch5 in Fig. 32d). The abdominal chordotonal organs of the larva become the pleural abdominal chordotonal organs eventually formed in the adult fly.

The first neuron to appear in morphogenesis (about 6h) belongs to the dorsal cluster. Then an lch5 scolopidial neuron appears, followed by the v'ch1 neuron and one or more ventral es neurons. Over the next hour, more cells are added to the clusters in the dorsal-ventral sequence. By stage 12 (9h) each cluster has attained its full complement of sensory neurons. Axogenesis begins shortly after the neurons appear, so that the first dorsal es neuron establishes the peripheral pathway as a pioneer cell. Once it has established the pathway, the lch5 axon begins to grow toward it. These axons grow ventrally and meet an outgrowing central axon, to form the anterior pathway for the cluster (Ghysen et al. 1986). The posterior pathway is pioneered by the v'ch1 scolopidial neuron followed closely by other neurons of the ventral cluster.

10.4.2.2 Pupal morphogenesis Identification of degenerating neurons during metamorphosis has been problematic. In some cases developing adult axons grow along persisting larval nerves (Ghysen et al. 1986), but in other
cases some larval neurons persist through the late pupal stage (Lakes and Pollack 1990).

The formation of adult chordotonal organs is known only for the blowfly *Phormia regina*, where post-larval morphogenesis of leg chordotonal organs has been elucidated using HRP immunochemistry (Lakes and Pollack, 1990). At the prepupal stage of the larva, each imaginal leg disc contains 8 neurons (7 in *Drosophila*) derived from the embryo. One is a scolopidial neuron located on the dorsal surface of the disc, but this cell probably degenerates early in pupal development. As the leg disc enlarges into the limb bud during pupation, the first few adult sensory neurons arise, and by one day after pupation most of the neurons are present. By three days (half the pupal period) the adult pattern is formed.

The major chordotonal organ in the dipteran prepupal leg bud is the FeCO, which comprises at least 50 FeCO neurons in the distal end of the presumptive femur. Two hours after pupation, these neurons sprout axons in two fascicles, which grow to the limb base apparently without an established pioneer neuron nor with guidepost cells. By 7.5h a tarsal chordotonal organ appears and by 26h a tibial chordotonal organ is evident in the distal tibial segment of the bud. Meanwhile, the FeCO dendrites have all oriented distally towards the FeCO apodeme and begin to divide into proximal and distal clusters. The proximal cluster migrates to its final position near the trochanteral border. At 51h it appears as two recognisable scoloparia, one dense and one dispersed (Debaisieux, 1938). The proximal FeCO has thus decreased its total nerve length by 750 µm, apparently through axonal shortening. *Phormia* is the only known case in which the FeCO includes two widely separated organs. In adult *Drosophila* the FeCO lacks the distal cluster and comprises only two scoloparia from the proximal cluster.

10.5 **Morphogenesis of nerve pathways**

The developmental mechanisms by which chordotonal organ axons find their way to the CNS involves two processes: first, growing axons must navigate across surfaces in the absence of prior neuronal tissue cues, and second, axons contact and fasciculate with established pathways, probably using each other as contact guides towards targets (Bate, 1976; Jan and Jan, 1993). Insights into inherent principles of directed growth and targeting during development are based upon studies of grasshopper leg nerve morphogenesis. Two major concepts have emerged: pioneer neurones, and the guidepost hypothesis.

10.5.1 **Pioneer neurones and the guidepost hypothesis**

Pathway formation is initiated by one or a few early pioneer neurons (Bate, 1976) which differentiate from the embryonic epithelium and send the first axons toward the CNS. The mechanisms by which they establish the two main
leg nerve pathways from the periphery are proposed by the “guidepost hypothesis” (Keshishian and Bentley, 1983b).

1. **Filipodial exploration.** Extensive filopodia from pioneer cell bodies, and later axons, sample the epithelial environment and may locate guidepost cells.

2. **Cell to cell recognition.** Pioneer axons grow along a chain of immature neurons, which have distinctive surface features that allow pioneers to make selective junctions with them.

3. **Axonal contact guidance.** New axons grow alongside existing axons.

4. **Positional cell determination.** Immature neurons differentiate from the epithelium in strategic locations and at times that allow them to determine pioneer routes by acting as guideposts. The resultant pathways become the adult nerve routes.

The critical feature of the guidepost hypothesis is point (4), in which stereotyped nerve routes are created by a chain of guidepost neurons or “follower neurons”, serving to guide the pioneers centrally. The followers also serve as target points where newly arising chordotonal organ axons join pioneer tracts, thereby creating nerve branch points (Keshishian and Bentley, 1983a,b).

The following two sections show how the two main branches of the leg nerve (N5B1, N5B2), both of which contain chordotonal organ afferents, develop in the embryo.

10.5.1.1 **N5B1 development** In grasshopper legs the first pioneers are a pair of tibial epithelial cells, collectively labeled Ti1, which differentiate from the distal limb bud epithelium at about 30% of development and soon become displaced to the level of the tibia (Fig. 32b). They send axons bearing 75-100 µm long filopodia on their growth cones over the epithelial surface towards the limb base and into the CNS. The Ti1 neurons grow along a chain of non-axonic partially differentiated follower cells: the CT1 pair near the coxo-trochanteral margin and F1 and F2 in the proximal and distal femur. The follower neurons subsequently send axons centrally along the Ti1 axons to establish a fasciculated pathway by the time chordotonal organ axogenesis commences. Thus the first embryonic leg nerve (N5B1 in the adult) is established and maintained, including stereotyped branch points determined by follower neurons (Fig. 32b). The follower neurons F1 and F2 later become Type II multipolar stretch receptors (Keshishian and Bentley, 1983a).

The first chordotonal organ axons to arise are those of the FeCO. At 38% development they grow toward and directly contact the F1 cell body (demonstrated by TEM) before growing proximally along the Ti1 axons. Later the subgenual organ (SGO) axons sprout and project onto the Ti1 cell bodies (Fig. 32b) and grow along the Ti1 pathway. Occasional dye coupling has been observed between chordotonal organ axons and pioneer cells in embryos earlier than 50%
development, but never later (Keshishian and Bentley, 1983a; Lakes-Harlan and Pollack, 1993).

10.5.1.2 N5B2 development The other major leg nerve in the insect leg, N5B2, is a mixed motor and sensory nerve. It receives afferent projections from the tarsal chordotonal organ and is pioneered by a later set of neurons than is N5B1: Ta1 and Ta2 in the distal tarsus, and Ti2 in the tibia (Fig. 32b). The pair of Ta1 neurons eventually (45% development) become scolopidial neurons in at least one tarsal chordotonal organ. Ta1 and Ta2 generate the future N5B2a and N5B2b branches, respectively, which extend proximally through the tarsus and tibia before joining Ti2 in the proximal tibia to produce N5B2 (Keshishian and Bentley, 1983b).

10.5.1.3 Necessity of pioneer and guidepost cells The Ti1 pair of pioneer neurons have a purely developmental role and they degenerate after the SGO axons have joined the leg nerve at about 55% development (Kutsch and Bentley, 1987; Lakes-Harlan and Pollack, 1993). However heat shock and ablation experiments have shown that pioneer and guidepost cells are necessary for correct establishment of the peripheral nerve architecture. If Ti1 pioneer neurons are killed, axons of the SGO fail to grow through the femur from the tibia (Klose and Bentley, 1988). Furthermore if coxal guidepost cells Cx1 are killed, the pioneer axons themselves may not reach the CNS (Bentley and Caudy, 1983).

10.5.2 Non-guidepost nerve morphogenesis

Nerve morphogenesis of the wing-hinge chordotonal organ in the grasshopper (Schistocerca) involves two pioneer neurons, but no guidepost cells have been demonstrated (Heathcote, 1981). At about 40% development the first cell of the chordotonal organ forms filipodia and sprouts an axon, which follows that of the adjacent multipolar wing stretch receptor neuron. Both act as pioneers which grow along a peripheral epithelial ridge toward the CNS, without evidence of dye-coupling to possible guidepost cells. During this time, the neuron cell bodies migrate in another direction toward their ultimate attachment site 150 µm away. The result is that the axons trail behind and form a pathway that eventually becomes the medial body wall nerve, and which serves as a route for later chordotonal axons from the same organ.

In other insects, especially the Diptera, guidepost cells do not appear to be required for nerve morphogenesis. The larval chordotonal organs in flies depend upon the initial pioneers from the dorsal and ventral clusters (Fig. 32d) for pathfinding, but appear to follow the pioneer axons without intervening guidepost cells to mark nerve branch points or turning points (Ghysen et al. 1986; Jan and Jan, 1993). In pupal chordotonal organs, e.g. the FeCO of the blowfly leg, large numbers of simultaneously-growing axons appear to do their
own pathfinding without pioneer or guidepost cells (Lakes-Harlan and Pollack, 1993).

10.5.3 Development of central projections of chordotonal organs

The role of pioneer and guidepost cells in determining the projections of leg chordotonal axon projections within the CNS have been described in the grasshopper (*Chorthippus biguttulus*) (Lakes-Harlan and Pollack, 1993). Once the Ti1 pioneers enter the CNS (at 38% development), they grow towards the midline of the respective thoracic ganglion and then turn abruptly forwards near the ganglion midline. They continue to grow anteriorly to the brain, fasciculating (and dye-coupling) with pioneers from more anterior thoracic segments but without branching for the entire distance. Guidepost cells Fe1 and Tr1 take the course created by the pioneers, including the anterior turn, but they do not project farther than one neuromere rostral to their point of entry (42% development). Shortly thereafter, SGO and FeCO axons (distal scoloparium) enter the CNS along the nerve 5 pathway defined by the pioneers. The axons of neurons from the FeCO proximal scoloparium have not been stained because of their small diameter. There is no dye-coupling between chordotonal afferents and pioneer or guidepost cells. The SGO axons grow, without branching, about halfway towards the midline. Some have collaterals which project for a short distance rostrally in the area of the pioneer tract, but in no case is there any fasciculation of chordotonal axons with that tract. The FeCO axons follow a similar route, and although they produce filipodia and short collaterals, they develop no significant branches. These begin to develop only after 60% development, with secondary and higher order branches developing in later stages of embryogenesis. The neuropil projections of the chordotonal axons resemble those of adult CNS (see Section 8.5).

In systems that lack guidepost cells less is known about the development of central projections. In a grasshopper, the two pioneer neurons responsible for creating the nerve route for the wing-hinge chordotonal organ (the first chordotonal neuron and the wing stretch receptor neuron), each take a different route when they reach the CNS, suggesting that they respond differently to local guidance cues. The chordotonal axon enters the posterior end of the embryonic ganglion, dives ventrally and sends branches to the neuromere’s ventral neuropil as well as toward the next anterior ganglion. Subsequent wing-hinge chordotonal axons follow this basic pathway, which is retained in the adult (Heathcote, 1981).

10.6 Physiological consequences of delayed development in Orthoptera

Delayed chordotonal organ development in orthoptera apparently means that instars lack the full physiological sensitivity found in adult chordotonal organs. Additionally, in tympanal organs of tettigoniids and gryllids, major enhancements
of cuticular accessory structures associated with hearing only appear in the final moult to the adult stage. For example, crickets do not develop the thin transparent tympanal membrane in the tibial cuticle until the final (tenth) moult (Ball and Young, 1974). Bush crickets show a progressive development of the cuticular tympanal membranes and their outer cuticular covers. The thickness of the cuticle decreases from 25 µm in the third instar, to 10-15 µm in the penultimate instar, and finally to 5 µm in the adult (Rössler, 1992b). The prothoracic trachea and spiracle act as an acoustic horn in tettigoniids, and the diameter of the spiracle enlarges dramatically in the final moult to adult (Rössler, 1992b). All the above changes would be expected to enhance acoustic sensitivity in the transition from last instar to adult.

In both tettigoniids and gryllids, threshold sensory tuning curves of pre-adult instars (ninth instar, cricket (Ball and Hill, 1978); fourth instar, bush cricket (Rössler, 1992a,b)) have the same shapes as those of adults, including identical peaks. Differences occur in the absolute threshold levels. Ninth instar crickets are 52dB (approximately 400-fold) less sensitive than adults, whereas fourth and fifth instar bush crickets are some 30dB (approximately 32-fold) less sensitive than adults. The reason for similarity in tuning curves apparently lies in a similar size of (a) cap cells and (b) area of contact of the crista acustica with the tectorial membrane and acoustic tracheal chambers in instar and adult organs (Rössler, 1992a). Developmental processes therefore yield tuning of the tympanal organ primarily based upon chordotonal sensillum size. A behavioural interpretation is that instars do not depend heavily upon a sense of hearing, since they are sexually immature and acoustic communication in orthopterans is mostly used by adults for mate attraction and territoriality.

11. Genetics and molecular biology

Genetic studies of chordotonal organs have occurred within the much broader scope of discovering how genes control the unique and stereotyped structure of the nervous system. *Drosophila* has been the model species, because the developing embryo can be easily subjected to genetic manipulation, and the formation of specific neuronal structures can be analysed. Earlier reviews on developmental genetics of the nervous system (but not specifically chordotonal organs) in *Drosophila* include those by Campos-Ortega (1985), Campos-Ortega and Hartenstein (1985), Ghysen and Dambly-Chaudière (1989), Campos-Ortega and Jan (1991) and Jan and Jan (1993, 1994).

Genes that control the development of chordotonal organs and other sense organs can act broadly to determine major domains, gradients and polarity of epithelial sheets that ultimately produce sensilla, or specifically to determine the type and fate of each cell within a sensillum (Jan and Jan, 1993, 1994). Most knowledge is available for cuticular hair and campaniform sensilla, with less for chordotonal organs and least for multipolar (Type II) sensilla (but see
Only a few studies have analysed the structure of genes that produce clearly identified products, such as β1-tubulin (Buttgereit et al. 1991). More commonly, the effects of single genes on sense organ development are studied through mutation experiments, and their products are usually not identified. Virtually nothing is known about the genetic control of chordotonal organ or cuticular sensillum physiology, although it is thought that “neuronal precursor” genes may determine the physiological characteristics unique to neurons (Jan and Jan, 1993).

Some genes that are active early in sense organ development appear to be involved in development of other tissues as well, and seem to always act in concert with members of a “functional cassette”. Thus neurogenic genes (Section 11.1) appear to serve the general function of generating a specific fate for one cell from an equivalent group of cells, whether it is in neurogenesis, myogenesis or oogenesis. In another example, the regulatory genes da, scute, emc and deadpan not only specify early neuronal position and sense organ potential, but also regulate sex determination through control of the ratio of X chromosomes to autosomes (reviewed by Jan and Jan, 1993).

Hypotheses about the function of specific molecules in scolopidia have been developed by using the electron microscope to locate antibodies (e.g. Fig. 2) against gene products expressed in chordotonal organs (e.g. Wolfrum, 1990).

### 11.1 Model for genetic determination of sensilla

Experiments with *Drosophila* have led to a working model, proposed by Ghysen and Dambly-Chaudière (1989) and modified by Jan and Jan (1993, 1994), which stresses progressive determination of epithelial cells in the formation of insect sense organs, including chordotonal organs. The scheme, summarised in Table 6 and Fig. 33, depicts sequential steps in the process, but two qualifications must be added: (a) there is probably extensive feedback between steps, and (b) some genes are expressed in more than one step.

The function of the *prepattern genes* is to set up dorso-ventral gradients and antero-posterior segmentation domains in the developing blastoderm of the early embryo. This provides early positional cues for the focal action of later steps in neuronal determination. Some prepattern genes also act at a later stage. For example, *rhomboid* may function as a neuron type-selector gene as well as a prepattern gene (Bier et al. 1990; Jan and Jan, 1993).

When *proneural genes* are turned on in certain clusters of cells in the above domains, they endow the potential to become neuronal precursor cells. The expression of proneural genes provides positional information that is apparently used for later differentiation. Thus chordotonal organ precursors arise in the posterior compartment of the embryonic segments whereas cuticular sensilla arise from the anterior compartments. Positional reading of gradient cues in this process is accurate to about 10% along one dimension. Apparently the patterns of proneural gene expression are important cues for
Table 6. Sequential model for the action of known genes that determine epithelial cells to become chordotonal organs (after Ghysen and Dambly-Chaudière, 1989; Jan and Jan, 1993, and Y. N. Jan, personal communication)

<table>
<thead>
<tr>
<th>Gene type</th>
<th>Prepattern genes</th>
<th>Proneural genes</th>
<th>Neurogenic genes</th>
<th>Neuronal precursor genes</th>
<th>Neuronal precursor type selector genes</th>
<th>Cell division and cell fate genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene name</td>
<td></td>
<td>da, ato</td>
<td>N, Dl, bib, neu, E(spl), mam</td>
<td>pros</td>
<td>cut</td>
<td>numb, N, Dl, ttk, cyclin</td>
</tr>
<tr>
<td>Function</td>
<td>Provide positional cue</td>
<td>Endow group of cells with potential to become neuronal precursors</td>
<td>Single out cells to be neuronal precursors and inhibit neighbours</td>
<td>Confer neuronal properties?</td>
<td>Specify neuronal type</td>
<td>Control cell division and cell fate genes</td>
</tr>
</tbody>
</table>

Unclassified genes that affect the number of chordotonal organs in local regions: pnt, rho, spz, S

Abbreviations: ato, atonal; bib, big brain; da, daughterless; Dl, Delta; E(spl), enhancer of split; mam, mastermind; neu, neuralized; N, Notch; pnt, pointed; pros, prospero; rho, rhomboid; spz, spitz; S, Star; ttk, tramtrack
Fig. 33. Progressive specification of chordotonal organ cell fates. The model is a modification of that for cuticular sensilla (Jan and Jan, 1994), based upon Ghysen and Dambly-Chaudière (1989). In sequence, proneural genes are expressed in clusters of hypodermal cells (proneural cluster cells, shaded) lining the cuticle, and confer those cells with the potential to become neuronal precursors. These cells then compete with each other so that only one cell (black) is singled out to express a high level of proneural gene and develop into a neuronal precursor, whereas the rest adopt the alternative epidermal fate. This singled-out cell then expresses neuronal precursor genes, which probably control neuronal precursor-specific differentiation, and neuronal type-selector genes, which confer neuronal identity. The precursor then goes through stereotyped cell divisions and produces a fixed number of progeny cells (compare with Fig. 30). Using both cell-cell interaction and intrinsic mechanisms, these progeny cells assume different (usually four) fates: accessory cell, attachment cell, scolopale cell and sensory neuron. Together they form a scolopidium. See Fig. 30 for different cell fates in cuticular sensilla. Modified from Jan and Jan (1994), with permission.

positional reading in the embryonic epidermis, rather than compartmental identity of the cells (Huang et al. 1994).

The most intensely studied proneural genes in Drosophila comprise the four-gene achete-scute complex, which is required for the formation of cuticular sensilla (and thus not included in Table 6), and daughterless. The ubiquitously expressed protein product of daughterless combines with achete-scute protein in the proneural cell clusters to bind to DNA and
appears to regulate transcription of target genes that initiate neuronal precursor development (Brand and Campos-Ortega, 1989; Jan and Jan, 1993, 1994). A similar mechanism appears to operate for chordotonal organ neuronal precursor cell clusters, but instead of *achete-scute* genes, the proneural gene product of *atonal* is required to combine with *daughterless* protein (Jarman et al. 1993, 1995). However, the specification of chordotonal organ precursors by the proneural gene *atonal* differs from external sensilla specification in one way: external sensilla arise from solitary precursors whereas chordotonal organs arise from clustered precursors (Jan and Jan, 1994). It appears that in both cases a single neuronal precursor is specified from the proneural cluster, but in chordotonal organs that precursor induces the formation of a chordotonal organ cluster which later develops into the chordotonal organ (Jarman et al. 1993, Jan and Jan, 1994). This presumably is the way scoloparia develop in the majority of chordotonal organs seen in insect appendages (see Section 3).

In the grasshopper *Schistocerca* the gene *Lachesin* is expressed in proneural clusters early in neurogenesis. Its product is an Ig cell surface protein which may be involved in cell recognition and adhesion, and the gene itself is thought to be a target for regulation by nuclear proneural genes (Karlstrom et al. 1993).

*Neurogenic genes* operate on the proneural cell cluster to select a small subset of cells that become neuronal precursors. These genes have not been studied specifically for chordotonal organ formation, although the following mechanism is likely to apply to all sense organs of insects. The process of singling out neuronal precursors is thought to involve mutual inhibition, in which neurogenic genes alter graded differences among proneural cells into an all-or-none difference between one cell (which becomes the neuronal precursor, SMC, also known as SOP, sensory organ precursor), and the remainder of the cells, which become epithelial cells (Goriely et al. 1991; Campos-Ortega and Jan, 1991). Six neurogenic genes are known: *Notch, Delta, Enhancer of split complex, mastermind, neuralized* and *big brain*. Deletion of any of these leads to hypertrophy of all types of sense organs as well as CNS tissue, although each seems to have different effects and some are required for final specification of cell fate whereas others are not (Jan and Jan, 1993, 1994).

*Neuronal precursor genes* are thought to endow the neuronal precursor cells with properties that distinguish them from neighbouring epidermal cells (Jan and Jan, 1993). This includes not only developmental properties (enlargement, delamination, division pattern) but also specific neuronal properties (axonal and dendritic differentiation, electrogenic ion channel and transmitter/receptor molecule production). Some genes are expressed universally in neuronal tissue (*deadpan, asense, scratch* and *prospero*), whereas others are expressed primarily in sense organs (*couch potato*). The gene *pros* affects the axonal pathways of chordotonal organs and most other sense organs.
Neuronal precursor gene products are thought to be regulatory in function, based upon their structure and prosthetic groups (Vaessen et al. 1991; Bellen et al. 1992; Bier et al. 1992; Jan and Jan, 1993).

The neuron-type selector genes specify the sense organ type that will arise from the SMC. Two genes are known, one of which selects chordotonal versus external cuticular sensillum type (cut), whereas the other (pox-neuro) selects singly- versus multiply-innervated external sensillum type (Jan and Jan, 1993). In normal larvae and adults, cut is expressed in external sensilla precursors, but not in chordotonal organ precursors, and it is required for acquisition of correct identity in external cuticular sense organs (e.g. campaniform and hair sensilla). The cut gene acts as a binary switch: if it is deleted, the SMC produces a chordotonal organ (Plate 2a,b). If it is forcibly expressed in the chordotonal organ SMC, an external cuticular sensillum develops. Thus the transformation is the result of loss of cut function in the external sensillum precursor cells (Bodmer et al. 1987; Blochlinger et al. 1988, 1990, 1991). The situation is not so clear in the CNS. The projections of external sensillum neurons that have been transformed to chordotonal neurons by mutation of the cut gene are rather variable: some resemble wild-type external sensillum projections, others resemble chordotonal projections, whereas others do not resemble either type. Although the cut gene affects sensory projections, it appears not to act as a simple switch of axon growth pattern within the CNS (Merritt et al. 1993).

The cut locus is large and genetically complex, as are the effects of the cut mutation (Bodmer et al. 1987). The sensory neuron dendrite is transformed from a curved, tapered shape, characteristic of hair sensilla neurones, to a short, straight shape characteristic of chordotonal neurones. The heavily-staining “dot” at the sensillum tip (undoubtedly the tubular body of Thurm, 1965) is transformed into a scolopidium in the cut mutation. In addition to morphological changes, there are organ-specific antigen changes. Chordotonal-specific staining with 49C4 and 58C12 antibodies occurs in the neuron and accessory cells of the transformed hair sensilla (Bodmer et al. 1987).

In contrast to the essentially complete transformation of external sensilla in fly larvae with the cut mutation, adult gymnandromorph flies with mosaic cut deletions differ with regard to external sensory structures. Although the transformed sensilla resemble chordotonal organs internally, they retain bristles and hairs externally, which are reduced in size compared to equivalent wild-type structures (Plate 2c,d). The hybrid sensilla of cut mutants may lend credence to suggestions of homology between external cuticular sensilla and chordotonal organs (see Section 12.5), although it is not clear whether the accessory cells that form the bristle and socket are the same (homologous?) cells that comprise the scolopidium (Bodmer et al. 1987). An electron microscopical study of the hybrid sensilla would shed light on this question, and determine whether the bristles are innervated.

Four additional genes are known to act on chordotonal organs: rhomboid, spitz, pointed and Star. Although they may act as type selector genes, their
classification and mechanism of action are not clear (Jan and Jan, 1993). At least rhomboid and spitz are known to affect chordotonal organ development at the cell precursor stage. Mutation of any of these genes results in loss of subgroups of chordotonal organs; thus only three of the five lateral chordotonal organs remain in abdominal hemisegments in mutants. The genes also act early in the pattern setting stage and may therefore be involved in chordotonal organ specification at several developmental stages.

Cell division and scolopidium cell fate genes control interactions and fate specification of the progeny of the SMC. In external bristle sensilla, Notch is known to be involved in cell to cell interactions, probably as a membrane receptor protein (Hartenstein and Posakony, 1990), whereas Delta protein is a ligand, so it is probable that in all sense organs, daughter cells communicate with each other to assume their correct fate (Jan and Jan, 1993; 1994). This communication appears to involve an extrinsic mechanism (Notch/Delta) and an intrinsic mechanism (numb), discussed more fully in Section 11.3.1. For chordotonal organs, Notch is probably required to specify scolopidium cell fate (Uemura et al. 1989), whereas numb interacts with Notch and Delta to control the asymmetric cell division process (Jan and Jan, 1994). The numb gene is essential for the multiple asymmetric divisions leading to correct chordotonal organ formation, since numb mutants have incomplete chordotonal organs that lack sensory neurons and show duplicated cap cells (Uemura et al. 1989). Recent work suggests that a previously-identified gene, tramtrack (ttk; thought to be putative transcriptional repressor involved in eye and cuticle development), is required to enable the numb protein to endow the proper cell fate on SMC daughter cells.

11.2 GENE STRUCTURE AND EXPRESSION CONTROL

The structure and expression of several chordotonal organs genes have been characterised in Drosophila and Schistocerca (grasshopper). Some are genes expressed almost exclusively in chordotonal organs whereas others are expressed generally in neural tissue.

The gene numb has been isolated from Drosophila and mapped. The 3.5 kb zygotic transcript contains an open reading frame which encodes a primary protein of 556 amino acids (61 kDa). The gene contains a large intron between the first and second exons, into which inserts a transposon. A highly basic region and a putative zinc finger region are suggestive that the numb protein binds to nucleic acids (Uemura et al. 1989).

Genes coding for immunoglobulin superfamily proteins have been cloned from grasshopper and Drosophila nervous system, where their expression product is found primarily on axons. The product of the Lachesin gene, lachesin, is expressed at 28-30 % development in differentiating chordotonal organs of limbs (femoral chordotonal organ) and body (auditory, pleural and sternal chordotonal organs) (Karlstrom et al. 1993). A cloned 2kb region of
this gene in the grasshopper contains an open reading frame coding for a 349 amino acid product. Another immunoglobin-coding gene expressed in chordotonal organs is \textit{Fasciclin I} (Meier and Reichert, 1990). The sequenced cDNA clone from grasshopper is 3.2kb in length and contains both 3' and 5' untranslated regions. The open reading frame codes for a protein of 638 amino acids (70 kDa), which is consistent with the expected molecular mass of fasciculin I (Zinn \textit{et al}. 1988). The homologous gene isolated from \textit{Drosophila} is 3.0 kb in entirety and encodes a protein almost identical to that of the grasshopper. The fasciculin family of proteins (only fasciculin I is found in chordotonal organs) are expressed later than lachesin, and function as adhesion/recognition molecules on the surfaces of outgrowing axons during chordotonal organ development (Bastiani \textit{et al}. 1987; Meier and Reichert, 1990). Expression control of these genes is unknown.

Two studies have examined the control of gene expression for tubulin production, using the lacZ reporter gene technique. Both \(\alpha\) - and \(\beta\)-tubulin proteins are encoded by multi-gene families, and are involved in the construction of microtubules expressed in scolopidial cells (and in other tissues) during development. The genes for tubulins function as effector rather than as regulator genes (Buttgereit \textit{et al}. 1991).

The \(\alpha 2\)-tubulin gene extends from nucleotide positions at -2.2kb to +2.7kb from the transcription point, and includes two introns. The DNA sequence between -2.2kb and -223 bp is necessary for \(\alpha 2\) expression, while the larger sequence from -2.2kb to +448bp appears necessary and sufficient to account for all normal tissue-specific expression and timing of \(\alpha 2\)-tubulin in embryonic development. The gene is expressed in only two tissues: chordotonal organs (including dorsal, ventral and lateral groups in larvae) and testes (Bo and Wensink, 1989).

Another tubulin isotype, \(\beta 1\), is expressed in all early embryonic tissue of \textit{Drosophila} and later in CNS, chordotonal tissue and muscle apodemes. The structure and regulatory action of the \(\beta 1\) gene has been studied as a model for understanding the functions of regulatory sequences in introns of a continually expressed gene (Buttgereit \textit{et al}. 1991). Using lacZ fusion genes, it was established that the necessary DNA of the \(\beta 1\)-tubulin gene includes a -2.2kb element upstream from the transcription initiation site, an element from the start site to +0.44kb, an intron from +0.44kb to +2.5kb, and a second exon 315bp downstream from +2.5kb. The intron is especially important for chordotonal \(\beta 1\) expression. Experiments with intron-deleted fusion genes showed that the intron contains two types of enhancer elements: one that autonomously confers cell type specificity and is able to cooperate with a heterologous promoter, and another that requires upstream elements of the \(\beta 1\)-tubulin promoter to enhance or activate correct temporal and tissue-specific expression (Buttgereit \textit{et al}. 1991).

The \(\beta 1\)-tubulin gene is one of the first structural genes to be turned on after cell fate has been determined. It must be under at least indirect control by
neuron type selector and/or cell fate determination genes discussed above, although details of control interactions are lacking.

Studies of tubulin gene expression suggest that genes are turned on differentially in cells of chordotonal organ tissue. In embryos, α2-tubulin occurs in the support cells of scolopidia, whereas in the third instar larva it occurs in axons and nerves (Matthews et al. 1990). It is not known whether α2-tubulin expression differs within the component cells of the pupal and adult scolopidia. A related issue concerns differential expression of ratios of tubulins in scolopidia. In other α2 isotypes there are large, non-conservative substitutions in the carboxy-terminal region which are likely to be involved in regulation of microtubule assembly and in interactions with microtubule-associated proteins (MAPs) (Bo and Wensink, 1989). Such substitutions imply differences in the expression product. Future work could use genetic manipulation and ultrastructural analysis of tubulin expression to explore the potential differences in tubulin, MAPs and microtubule structure, in chordotonal scolopidia known to have different physiological properties. The final step, of establishing the physiological response properties of different chordotonal sensilla in Drosophila, could allow insight into the mechanisms of genetic control and differentiation of sensory properties in these sophisticated sense organs.

11.3 MOLECULAR BIOLOGY OF GENE PRODUCTS

Progress in isolating and characterising genes and their products has been facilitated by the ease of combining genetic and molecular biological techniques in Drosophila and the grasshopper (reviews by Boyan and Ball, 1993; Jan and Jan, 1993, 1994). The genes reviewed in the following occur in chordotonal organs and other insect Type I sensilla, except where noted.

11.3.1 Regulatory proteins

Many of the genes that control chordotonal organ differentiation are transcriptional regulators. They code for proteins that have characteristic domains such as zinc fingers or helix-loop-helix (HLH) structures that allow the proteins to bind to DNA and to regulate the transcription of DNA into RNA. These proteins are called transcription factors because they either activate or repress transcription. Other regulatory proteins appear to be intrinsic membrane receptors involved in signal transduction pathways used during sense organ development.

Interactions of the proneural gene products of daughterless (da), atonal (atn), and achaete-scute complex (ac and sc are only in external cuticular sensilla) have been extensively studied. All products are nuclear protein dimers with an HLH structure and an adjacent basic domain on each monomer. The da protein is ubiquitously expressed and is capable of binding with DNA.
The regionally-expressed $atu$, $ac$ and $sc$ proteins are able to form heterodimers with $da$ protein and subsequently bind to DNA and regulate transcription in vitro (Cabrera and Alonso, 1991). Thus, in sense organ proneural genes, $da$ protein appears to act as an available cofactor for heterodimer formation once $atu$ protein is expressed; this dimer controls the regulation of downstream target genes to initiate neuronal precursor cluster formation. In the $ac-sc$ (and by implication $atu$) pathways, a negative regulation of the localised HLH protein is achieved by the gene extramacrochaete ($emc$). Because the $emc$ product lacks the basic domain for binding to DNA, it blocks transcriptional regulation when combined with $ac-sc$ proteins, and thus it regulates their concentrations. The observed expression pattern of $emc$ is complementary to that of the $ac-sc$ genes, supporting the notion that the balance of these gene products determines the competence and distribution of cells that form neural precursors in the embryonic ectoderm (Jan and Jan, 1994).

Neurogenic gene products (e.g. Notch and Delta, Table 6) are characterised by repeats of EGF-like (Epidermal Growth Factor) domains. They belong to a group of proteins involved in membrane-receptor-mediated signalling pathways. Notch is a membrane protein which is probably the receptor, whereas Delta is a putative ligand. The overall functions of neurogenic genes in chordotonal organs are to: (a) single out founder neural precursor cells, through lateral inhibition; and (b) induce the specification of clustered neural precursors from neighbouring cells in the proneural cluster (see Section 11.1). The cell-cell interaction responsible for lateral inhibition is probably mediated by the Notch/Delta proteins (likely to operate in chordotonal organs, but only confirmed in external sensilla). Recruitment by induction appears to be mediated by another group of genes specific to chordotonal organs: the spitz gene group ($spitz$, rhomboid, pointed, Star). Their products are closely associated with EGF membrane receptors, and represent another intercellular signalling pathway for chordotonal organs. The $spitz$ protein resembles EGF and is probably a ligand; the rhomboid product is a transmembrane protein that probably acts as a cofactor in the signalling process (Jan and Jan, 1994). Both EGF-like groups of proteins make up an extrinsic mechanism for cell-cell interaction in specifying asymmetry in cell fate of SMC daughter cells.

The neuronal precursor gene, prospero, in both chordotonal and cuticular sensilla, encodes a large nuclear regulatory protein with several stretches of homopolymeric amino acids expressed transiently in neuronal precursors (Vassein et al., 1991; Bier et al., 1992).

Amongst the cell division and cell fate genes (Table 6) the numb protein and the Notch/Delta proteins belong to two signalling pathways (extrinsic and intrinsic) that interact to generate asymmetric cell division in sensilla formation (Jan and Jan, 1994). In chordotonal organs the numb gene is required for correct fate determination in the progeny of the scolopidial precursor cell (SMC). The product of numb is a membrane-associated protein (61 kDa, 556 amino acids) unevenly expressed as a crescentic patch on one side
of the SMC plasma membrane. The first SMC division therefore results in an asymmetric distribution of numb protein in the daughter cells (Uemura et al. 1989). The numb protein is likely to function in a signal transduction pathway and represents an intrinsic mechanism of cell-cell interaction in the chordotonal cell lineage (Jan and Jan, 1994).

The numb protein places a bias on the Notch/Delta protein concentration balance in daughter cells of the SMC. The Notch/Delta system is a positive feedback inhibition mechanism in which a slight concentration imbalance of one or the other protein in the first two daughter cells will lead to amplification of the inhibitory influence of one cell on the other: a higher concentration of Notch protein will lead to a progressive decrease in Delta protein and drive the two cells toward their different fates. Jan and Jan (1994) propose that this system may not be reliable within the timed events of embryogenesis: they propose that the addition of numb product as a suppresser of Notch would ensure an accelerated Notch/Delta interaction due to the strongly asymmetrical distribution of numb product. Verification of the hypothesis requires elucidation of the components of the two pathways. Furthermore, tramtrack (ttk) products are required to generate the effects of numb (Guo et al. 1995). ttk codes for 69 kDa and 88 kDa polypeptides, which have different pairs of zinc fingers and a common N-terminal region containing a conservative BTB domain (protein-protein interaction sequence) (Godt et al. 1993). Both bind to DNA and are expressed in embryonic cells of the peripheral nervous system. They are thought to act as downstream transcription factors which execute the command of asymmetrically-distributed numb protein by regulating cell-type specific gene expression in one of the daughter cells (Guo et al. 1995).

The regulatory nuclear protein encoded by the cut gene has a homeo domain. The predicted product from cDNA sequences is 2175 amino acids, and Western blots show two proteins with relative molecular masses of 320 and 280 kDa (Bodmer et al. 1987; Blochlinger et al. 1990). It is thought that the cut product acts as a transcriptional regulator of expression of downstream differentiation, and is found in all cells of external cuticular sensilla. This action takes the form of a sensitive binary switch expressed in the neuronal precursor cell: if it is expressed, the cell produces a cuticular sensillum; if it is not expressed, a chordotonal sensillum is produced (Blochlinger et al. 1990; Jan and Jan 1993).

11.3.2 Structural proteins

Structural proteins in chordotonal organs have been discussed in studies of gene characterisation, developmental pathway formation, and functional analysis of ultrastructural components in scolopidia, but little is known about how structural proteins contribute to morphology and sensory function in chordotonal sensilla.

Tubulin is the primary component of microtubules, and therefore plays a
pivotal role in many developmental processes. α-tubulin in *Drosophila* is produced by a group of 4 genes, each coding for a specific isotype. Matthews *et al.* (1990) suggest that the presence of one of these isotypes (α85E-tubulin) in attachment cells of scolopidia may be related to these cells’ requirement to take on an elongated shape during development. In *Drosophila* this α-tubulin isotype is restricted to only a few elongate cell types. In chordotonal organs the different tubulin isotypes presumably interact with other proteins (e.g. MAPs) to produce a range of cytoskeletal structures with different functions in each cell type. For example, although microtubules occur in sensory neuronal dendrites, the distal ciliary segment, scolopales, scolopale cells and attachment cells, only the latter stain positively for α85E-tubulin (Matthews *et al.* 1990). Other α-tubulins, as well as various β-tubulin isotypes presumably comprise the non-staining microtubules in the remaining cases. Attachment cells of adjacent scolopidia should be examined for differences in microtubule composition in an effort to explain how they can have different physiological response properties (see Section 5).

Several fibrous proteins have been detected in scolopidia using a combination of immunocytochemistry and electron microscopy (Wolfrum, 1990, 1991a,c, 1992). Filamentous actin reacts with phalloidin, and occurs as bundles in the scolopale, closely associated with microtubules (Wolfrum, 1990). Myosin S1-decoration showed that actin filament polarity is unidirectional, suggesting that the actin has a stabilising rather than contractile role. Tropomyosin is co-localised with actin in the scolopale bundles in both light and electron micrographs (Wolfrum, 1991c). In muscle, tropomyosin is a rod-like protein that binds along the helical groove of the filamentous actin molecule; presumably it binds in similar fashion in scolopales. The role of tropomyosin in muscle is to (a) stabilise actin filaments by promoting actin assembly, preventing disassembly and increasing rigidity, and to (b) block the myosin binding site on actin through a Ca²⁺ mediated pathway. The absence of myosin in scolopidia suggests that the role of tropomyosin might be one of variable regulation of actin rigidity through local Ca²⁺ concentration (Wolfrum, 1991c).

Scolopidia also contain the fibrous proteins centrin and α-actinin, both of which are co-localised in the ciliary roots (Wolfrum, 1991a). In muscle, α-actinin anchors actin filaments to each other (forming the Z-line in sarcomeres) as well as to fine non-actin elastic filaments such as titin and nebulin (reviewed in Wolfrum, 1992). A similar role could occur in scolopidia. Centrin is a phosphoprotein with molecular mass of 20kD and is a member of the EF-hand superfamily of calcium-binding proteins. The presence of centrin, or a centrin-like molecule, raises the possibility of active Ca²⁺ mediated contraction of ciliary roots, inasmuch as centrin is known to be largely responsible for the Ca²⁺ regulated motility of flagellae in green algae (Wolfrum, 1991a). The above discoveries open up an array of experimental opportunities to pursue the roles of these structural proteins.
Most of the immunoglobin superfamily proteins identified in chordotonal organs are involved with establishing axonal pathways in the developing nervous system. They serve adhesive or recognition roles in laying down initial axon tracts or creating axonal fascicles. These proteins are glycosylated extrinsic membrane-associated proteins. The 70 kDa fasciculin I protein in grasshopper contains a signal sequence leader characteristic of secreted or membrane-bound proteins, as well as 6 glycosylation sites (4 in *Drosophila*) and 4 homologous domains of 150 amino acids each. Zinn *et al.* (1988) proposed a model for the tertiary structure of fasciculin I in which the protein comprises two dimers with binding sites at each end specific for ligands on apposing cell surfaces. Fasciculin I thus acts as a bivalent bridge between cells that express the ligands. Fasciculin I is expressed on developing axons and cell bodies of grasshopper auditory organ, pleural and wing-hinge chordotonal organs, and subsets of certain CNS tracts. In chordotonal organs it is thought to hold clusters of neuronal somata together during the migration stage of embryogenesis, and to promote fasciculation of each outgrowing sensory axon bundle and its incorporation as a fascicle into the intersegmental nerve (Bastiani *et al.* 1987; Meier and Reichert, 1990).

The 38 kDa glycoprotein lachesin also contains a leading signal sequence and no transmembrane domains. It contains a glycosyl phosphatidylinositol moiety linked to a hydrophobic C-terminal sequence, which provides strong evidence that it is an extrinsic membrane-associated protein. Based upon its primary structure, lachesin is most similar to the group of vertebrate and invertebrate adhesion molecules (TAG-1, F11, L1 and NgCAM) which share in common one variable (V) and two constant (C-2) type Ig domains. As indicated in Section 11.1, there is evidence that lachesin is not only involved in cell (particularly axon) adhesion during later phases of chordotonal organ development, but also serves as a cell surface recognition molecule involved in early preneural cluster specification (Karlstrom *et al.* 1993).

Glionexin is a large glycoprotein (265 kDa) found exclusively in insect mechanoreceptors, including chordotonal organs. Its glycosylated structure resembles a small proteoglycan (Meyer *et al.* 1987). Rather than being expressed in the cells of the scolopidia, it appears to be deposited by glial cells into the extracellular matrix (ECM) surrounding scolopidia (Field *et al.* 1994). There is evidence that it acts as a surface adhesion molecule in developing embryos (M. R. Meyer, pers. comm.). It may also serve as an ionic “sponge” to provide cationic homeostasis in sensory neuronal spike-generating regions, and an intercellular glue to provide mechanical stability in adult chordotonal organs (Field *et al.* 1994).

One further molecule detected in chordotonal neurons, as well as those of PNS and CNS tissue, is a membrane glycoprotein that reacts with antibodies to horseradish peroxidase (HRP). This protein allows global immunostaining of neuronal tissue (Jan and Jan, 1982; Snow *et al.* 1987).
11.4 Future research

The power inherent in genetic techniques should be combined with neurophysiological and electronmicroscopic techniques to more fully elucidate the functional ultrastructure of scolopidia. Since the small physical size of *Drosophila* is problematic, locusts may be more attractive for this approach. If, for example, locusts with genetic deletions for any of the expression products in scolopidia, but especially tubulins and MAPs, could be found, it would be possible to characterise ultrastructural changes together with physiological changes in sensory responses, since intracellular recordings can be made from single sensory neurons. A combination of genetic experimentation and immunogold analysis of expression at the ultrastructural level would greatly enhance this area of research.

Deletions for type-selector and cell fate genes, such as the *numb* mutant (Uemura et al. 1989), in which the neuronal component of chordotonal organs is deleted, could allow studies of resultant changes in central nervous circuitry and motor control mechanisms. Such studies should be combined with those of insect behaviour to thoroughly investigate motor control consequences of any specific sensory deletion. By focussing on mutations of proprioceptors such as chordotonal organs (which are involved in sensory feedback of joint position and movement), rather than of hair sensilla (e.g. Usui-Ishihara et al. 1995), it is more likely that insight into the genetic control of behaviour will be gained.

12. Evolution and homology

New inferences about the evolutionary derivation of chordotonal organs, and about homologies with other insect sensory tissues, have been based upon recent genetic and developmental advances. Several reviews have incorporated this approach to understanding homologous relationships within the insect nervous system (Boyan and Ball, 1993; Jan and Jan, 1994, 1995; Kutsch and Breidbach, 1994). Boyan and Ball (1993) presented a detailed insight into how lineage-related neurons can be used to understand structural and functional properties of the nervous system. Thus investigators can gain deep insight into neuronal circuitry, transmitter properties and synaptic connectivity by applying knowledge of specific relationships between bilaterally or serially homologous neurons. The reasoning applies equally well to chordotonal organs. Kutsch and Briedbach (1994) have developed a modern set of guidelines for establishing homologies in arthropod nervous systems, based upon genetic, immunohistological, pharmacological and developmental criteria, as well as traditional morphological criteria. They provided a broad review of known neuronal homologues, including sense organs, in nervous systems of arthropods.
12.1 CONCEPTS OF HOMOLOGY

In a phylogenetic sense, *homology* is defined as “a fundamental similarity due to inheritance from a common ancestral form”, that is, a synapomorphy. This definition has been used in various forms to compare similar organs amongst different species. Historically, morphological and developmental attributes have provided the basis for establishing interspecific homology (reviewed by Kutsch and Breidbach, 1994). More recently, homology has been limited in the strict sense to those structures that are descended from a common precursor cell or tissue (Arbas *et al.* 1991). In arthropod nervous systems, for example, neurons homologous between species are those that are descended from equivalent neuroblast precursors occupying equivalent positions within their respective tissue arrays and producing the same patterns of growth. Intraspecific comparisons encompass (a) *serial homology*, which is used to relate neurons derived from the same division of equivalent stem cells in sequential body segments, and (b) *bilateral homology*, which relates daughter cell neurons derived from equivalent stem cells on opposite sides of the same segment (Boyan and Ball, 1993).

It is important to consider ontogeny when attempting to establish homologies, since the evolution of morphology reflects the evolution of ontogeny (Kutsch and Breidbach, 1994). This is particularly applicable to insects, where the basic biochemical and structural attributes of neurons are established in the late embryo. But, especially in the Holometabola, neurons that may persist to the adult can change structure and function in the different life stages. By searching for homologies in the larval stages one is more likely to uncover common phylogenetic origins. Thus much recent work concentrates on embryological approaches backed up by molecular biological techniques to investigate homologies. The most dramatic example of the power inherent in this approach is seen in the current understanding of the ontogenetic relationship between chordotonal organs and cuticular sensilla (see Section 12.5).

Although modern definitions take advantage of genetic and immunological techniques to allow examination of molecular and developmental relationships amongst cells, this is not always possible in practice. Hence a combination of morphological, developmental and neurophysiological characteristics may also help to establishing homology. The following are useful in considering homologies amongst insect sense organs (Kutsch and Breidbach, 1994):

1. The morphology of the sensory dendrite and relationship to surrounding cells.
2. The course of sensory axons toward and within the CNS, as well as the relative position of axons within peripheral nerve bundles.
3. Similarity in unique physiological characteristics, such as non-spiking regions of neurons.
4. The production of different transmitter and/or neuromodulator molecules, as an aid in distinguishing different homologues.

5. Gene expression and genetic markers during development.

12.2 ANTENNAL CHORDOTONAL ORGANS

Two chordotonal organs occur in the antennae of nearly all insects: Johnston’s organ (composed of a ring of scolopidia around the inner periphery of the antennal pedicel), and the antennal connective chordotonal organ. Almost universally the scolopidia in Johnston’s organ are amphinematic, but in the mayflies (Ephemeroptera) the entire ring is composed of mononematic scolopidia (Schmidt, 1974). In other respects the ephemeropteran Johnston’s organ is little different from that found on other insects. Thus there is general agreement that the ring of mononematic scolopidia in Ephemeroptera is homologous with the insect Johnston’s organ (Schmidt, 1974; McIver, 1985; Bode, 1986). The reason for a major departure in mayflies from the normal scolopidial type found in Johnston’s organ is unclear, but the situation underscores the need to investigate basic physiological differences between the two types of scolopidia.

The Thysanoptera may present clues to the unusual lack of amphinematic scolopidia in the Ephemeroptera. In Johnston’s organ of thrips, the circular array of amphinematic scolopidia is divided into clusters (scoloparia) of five scolopidia each. Two scoloparia, however, contain a single mononematic scolopidium which seems fully integrated into Johnston’s organ and does not seem to belong to the more central connective chordotonal organ (Bode, 1986). Ultrastructurally the mononematic scolopidia appear to have lost the cuticular end thread that normally connects to the arthrodial membrane of the pedicel- flagellum joint. Instead each one is connected via an apical cap cell (attachment cell) close to the attachment sites of the cuticular end threads of neighbouring amphinematic scolopidia. There is no morphological discontinuity within the scoloparia containing mononematic scolopidia, and the basic number of five scolopidia per scoloparium is only met if the mononematic scolopidia are included. Furthermore, the caps of the two mononematic scolopidia resemble cut-off tubes rather than the uniformly dense cap normally seen in such scolopidia. Bode (1986) suggested that, in the course of evolution, some unknown process caused the two scolopidia to be transformed into the mononematic type, and that the same process operated more extensively to transform all Johnston’s organ scolopidia of Ephemeroptera into mononematic ones. To date, nothing is known about the genetic determinants of scolopidial type, but it is not unrealistic to expect that genes acting at the neuronal precursor type selector level, such as cut, could be responsible for the transformation. The difference between the two types must lie in genetic determination of the four-cell lineage of the scolopidium, rather than in fate-specific determination of the sensory neuron alone.
The connective chordotonal organ in the central axis of the pedicel of insect antennae is always composed of mononematic scolopidia. In some insects such as mosquitoes (Diptera), however, it is reduced to a small number of scolopidia (reviewed by McIver, 1985), and in thrips a central scoloparium is lacking. Although there are some grounds (e.g. a non-central position in the pedicel) for alternative interpretations, a single scolopidium in the thrip antenna is ultrastructurally distinct from those of Johnston’s organ and is mononematic. In thrips, and probably dipterans, the mononematic scolopidia appear to be homologous to the connective chordotonal organ of other insects. Although the organ appears to be reduced in thrips to a single scolopidium (Bode, 1986), there are no reports of it being absent altogether. An understanding of the evolutionary reduction of the connective chordotonal organ in the above insect groups must await neurophysiological investigation to elucidate differences in sensory responses of Johnston’s organ versus the connective chordotonal organ, and of mononematic versus amphinematic scolopidia.

12.3 Thoracic and abdominal chordotonal organs

12.3.1 Segmentally-iterated chordotonal organs

The most significant advance in understanding the evolution of thoracic and abdominal chordotonal organs stems from studies of their embryonic development in orthopterans and dipterans. The hemimetabolous grasshopper, representing the more primitive order Orthoptera, evolved at least 300 million years before the holometabolous fruit fly, in the highly specialised Diptera. Comparison of the development of the peripheral nervous system in these two insects has shown a) that the fundamental mechanisms for establishing the scaffolding of nerves and their sense organs are extremely conservative, and b) that there exists a high degree of serial homology amongst the sense organs along the segmented body axis (Boyan and Ball, 1993; Meier et al. 1991). The chordotonal organs show these principles most clearly.

In the grasshopper Schistocerca, sense organ precursors differentiate embryonically in each body segment once the initial nerve scaffolding is established by pioneer neurons (see Section 10). Three distinct clusters of chordotonal organs differentiate in each thoracic and abdominal segment along the body: the dorsal, lateral and ventral clusters. The axons of the dorsal and lateral clusters join into the intersegmental nerve, whereas axons of the abdominal ventral clusters join the segmental nerve. The best studied is the lateral cluster, which gives rise to three quite different chordotonal organs in the adult. In the thorax, it forms the wing-hinge chordotonal organ in the pterothoracic (wing-bearing) segments, whereas in the abdomen it forms the tympanal auditory organ in abdominal segment 1 and the pleural chordotonal
organs in the remaining segments (Meier and Reichert, 1990; Meier et al. 1991). The dorsal cluster gives rise to the multipolar wing-hinge stretch receptor (Heathcote, 1981) in the pterothoracic segments and possibly intersegmental stretch receptors in the abdominal segments, whereas the ventral cluster produces the sternal chordotonal organ and an uncharacterised ventral sense organ (Hustert, 1974; Meier et al. 1991). In the adult, the wing-hinge chordotonal organ is anchored to the wing articulation and monitors movement, whereas the tympanal organ is specialised to detect sound through cuticular modifications that form the ear. The pleural chordotonal organs detect stretch of the abdominal wall. As seen in Fig. 34a, serial homology is demonstrated for chordotonal sensilla that arise initially from similar developmental constructs but finally become specialised into monitors of different modalities such as stretch and hearing (Meier and Reichert, 1990; Boyan and Ball, 1993). In this case the advantage of applying embryological evidence in establishing serial homology is clearly seen: diverse chordotonal organs commonly share developmental mechanisms of neurogenesis, morphogenesis and axogenesis.

The development of the embryonic peripheral nervous system in Drosophila is remarkably similar to that found in the grasshopper (Fig. 34b), and there is a strong case for interspecific homology. The same dorsal, lateral and ventral clusters of sensilla are found in each thoracic and abdominal segment of the larva, and the same anatomical organisation of chordotonal organs and axonal projection patterns in the nerve scaffolding are retained (Ghysen et al. 1986; Meier et al. 1991). In homologous segments of the abdomen, for example, the lateral cluster gives rise to the lch5 chordotonal organ in Drosophila and the auditory and pleural chordotonal organs in Schistocerca. In addition, an identified cell from the dorsal cluster of the thorax serves initially as a pioneer for the intersegmental nerve and guide for the wing-hinge chordotonal axons, then later becomes the wing-hinge multipolar stretch receptor in the grasshopper; the homologous cell in Drosophila is thought to be the dh1 (dorsal trichoid hair) cell (Meier et al. 1991).

Arguments similar to those for serial homology in the grasshopper can be applied to thoracic and abdominal chordotonal organs in Drosophila. The homology of wing-hinge chordotonal organ with the auditory/pleural chordotonal organs in the grasshopper suggests that the thoracic homologue of lch5 in the Drosophila abdomen should be dch3 in the thorax. Even though dch3 is associated with the dorsal cluster and only contains 3 scolopidia instead of 5, evidence from cell lineage, precursor identity, timing of differentiation and axonal projection pattern support this homology. Furthermore, genetic evidence also points toward the same homology. Thus in rhomboid mutants, the 5-cell abdominal lch5 is transformed into a 3-cell chordotonal organ (Bier et al. 1990), and the engrailed locus exclusively deletes dch3 and lch5 without affecting other sensory cells of the lateral cluster (Hartenstein, 1987). The above approach demonstrates the power of combining developmental and
Fig. 34. Schematic summary diagrams of postulated homologies of chordotonal organs and cuticular sensilla between body segments within an insect species, and in the same body segment between insect species. **a.** Postulated segmental (serial) homology of all major sensory cell groups found in the body wall of the grasshopper embryo at 50% development. In the 2nd and 3rd thoracic segments (T2, T3) the wing-hinge chordotonal organs project via wing-hinge stretch receptor (diamond) and dorsal body-wall cell group (uppermost rounded oval) into the intersegmental nerve. In the 1st abdominal segment (A1) the auditory organ projects onto the intersegmental nerve. In the 2nd through 7th abdominal segments (A2-A7) the pleural chordotonal organs project onto the intersegmental nerve. In each abdominal segment, identified ventral receptor cell groups (ovals) project onto the anterior segmental nerve, which is pioneered by a single neurone (small circle) in the abdominal limb bud. Anterior to left, dorsal upwards. **b.** Interspecific homology for sense organ clusters of abdominal segments in the grasshopper (*Schistocerca gregaria*) and the fruit fly (*Drosophila melanogaster*). Abbreviations for *Drosophila* as in Fig. 32d. a, modified from Meier and Reichert (1990), with permission; b, modified from Meier et al. (1991), with permission.
genetic evidence to establish evolutionary relationships in nervous systems within and between species.

12.3.2 Tympanal organs

Tympanate hearing is widely distributed in at least six orders of insects (Hemiptera, Orthoptera, Neuroptera, Lepidoptera, Blattaria, Diptera), among which it has evolved in independent and diverse ways (reviewed by Michelsen and Larsen, 1985; Yager and Hoy, 1987; Fullard and Yack, 1993). Within the thorax and abdomen, tympanal organs are found on the prothorax of tachinid flies, lateral meso- and metathorax of notonectid and corixid bugs, dorso-lateral metathorax of noctuid moths, first abdominal segment of acridid grasshoppers and geometrid moths, and on the second abdominal segment of cicadas (Yager and Hoy, 1987; Yack and Fullard, 1993). The abundance of peripheral chordotonal organs serving a proprioceptive function at joints between sclerotised plates could easily have led to preadaptation for a hearing function (Fullard and Yack, 1993). For example, if the moth wing-hinge chordotonal organ was highly sensitive to the fine vibrations produced by the wings during flight warm-up, it might easily have evolved into a hearing organ to detect the ultrasonic cries of predatory bats once they began to exert selective pressure on moths. Furthermore, it is known that sounds induce cuticular vibrations that can excite chordotonal organs not specifically adapted for hearing. If during evolutionary change, external cuticular structures became thinner, or became intimately associated with enlarged internal tracheal structures, adjacent chordotonal organs could evolve into hearing organs.

This general scenario has been proposed for several tympanal organs and their respective non-hearing homologues. For example, the hind wing-hinge chordotonal organ of atympanate moths (e.g. Saturniidae) is thought to be the evolutionary prototype for the thoracic tympanal organ found in noctuid moths (Yack and Fullard, 1993). Detailed comparison of the sensory nerve (IIIN1b) branching pattern in the saturniid Actias luna and the tympanate noctuid Feltia heralis reveals close similarities, including the branch that bears the chordotonal organs in each species. The atympanate chordotonal organ contains three scolopidia, whereas the tympanate organ contains two. In both species, the chordotonal organ attaches to the peripheral membranous region under the hind wing alula by a thin elastic connective tissue strand. In Feltia the adjacent sclerotized cuticle has been reduced to an extremely thin tympanal membrane. The scolopidia of the two chordotonal organs are both mononematic and monodynal (i.e. each contains a single sensory neuron), which is characteristic of all tympanal organs in insects and presumably reflects a need for high sensitivity to the minute energy of acoustic stimuli compared to that of mechanical stimuli which move body parts. Electrical recordings from the sensory nerves of both chordotonal organs show that they each contain a
tonically active neuron unresponsive to sound, and 1-2 smaller neurons phasically responsive to sound. The case for homology of the chordotonal organs is thus built on morphological and electrophysiological evidence (Yack and Fullard, 1990), while differences between the two organs are mechanical and lie in non-neural structures such as the enlarged tympanal chamber, thinned tympanum and isolation of the tympanum from body movements (Fullard and Yack, 1993).

Ultrastructural examination of scolopidia from tympanal and atympanal chordotonal organs allowed Yack and Roots (1992) to suggest hypothetical changes, in addition to the mechanical ones noted above, involved in the transition from proprioceptive to hearing function. Mononematic, monodynal scolopidia are apparently the optimal type for hearing. In addition, characteristics of the tympanate condition include a decrease in length of the distal attachment strand, a decrease in microtubule density within the scolopidial attachment cells, and a loss of the sheath surrounding the strand. The resulting structure would result in a lower scolopidium threshold by stiffening the system in the tympanal strand. The mononematic, monodynal condition could provide enhanced mechanical coupling between structures in the evolutionary transition toward detection of minute and rapid tympanal membrane displacement (Yack and Roots, 1992; Ghiradella, 1971).

Other homologues for tympanal ears have been proposed in closely related atympanate insects. The prosternal hearing organ found in parasitic tachinid flies is thought to be derived from the prosternal chordotonal organ in the neck of atympanate flies such as the drosophilids and muscovids. The prosternal chordotonal organ of atympanate insects is thought to monitor neck movements (Hengstenberg, 1991), whereas the tympanal organ has many more scolopidia and monitors vibration of the anterior cuticular membranes of the ear, as a sound pressure receiver system. Both organs enter the frontal nerve of the thoraco-abdominal ganglion and project to all three thoracic neuromeres (Robert et al., 1994; Lakes-Harlan and Heller, 1992; see Section 8.4.3). Recently, one of the two paired prosternal chordotonal organs in the locust (Locusta) was shown to connect medially to the ventral cervical membrane, and to be sensitive to sound and gross neck movements; this system may represent a transition toward a tympanal organ (H.-J. Pflüger and L.H. Field, pers. comm.). In grasshoppers, comparative developmental studies show that the tympanal organ in the first abdominal segment of Schistocerca appears homologous to the pleural chordotonal organ found in the first abdominal segment of the more primitive atympanate species Heide amiculi. This interspecific homology supports Meier and Reichert’s hypothesis of segmental specialisation of serially homologous pleural chordotonal organs, which are essentially considered to be plesiomorphic (ancestral) (Meier and Reichert, 1990). The cyclopean tympanal organ of mantids is an exception to the above examples of atympanate prototype homologues. The organ projects to metathoracic nerve 7, which in cockroaches (in the same order, Dictyoptera)
supplies cuticular exteroreceptor sensilla of the basisternum and furcasternum, and is not known to supply any chordotonal organs. Furthermore the ganglionic projections of the sensory axons differ from those of all other tympanate insects (Yager and Hoy, 1987). Although a prototype homology is lacking for the mantid ear, an understanding of the evolution of this ear in the Mantodea is not. Within 330 mantid genera, five anatomical types of external cuticular modifications are associated with the ear. One of the types is associated with sensitive ultrasonic hearing, whereas another (Chaeteesidae) appears to be the plesiomorphic condition and is reflected in newly-hatched nymphs of other families showing more derived conditions in the adults (Yager, 1989).

12.4 LEG CHORDOTONAL ORGANS

12.4.1 Femoral chordotonal organ

In Orthoptera the femoral chordotonal organ consists of a proximal and a distal scoloparium (Fig. 8), both of which insert onto a common apodeme pulled by the tibia, but which are usually separated within the femur (Slifer, 1935; Burns, 1974; Field and Pflüger, 1989). The proximal scoloparium contains many very small sensory neurons, a form that is reminiscent of cicada abdominal chordotonal organs (Fig. 7b), whereas the distal scoloparium contains larger neurons characteristic of joint chordotonal organs. In the hind legs, the FeCO may differ from this plan, as there appears to be a variable degree of fusion of the two scoloparia throughout the ensiferan orthopterans (Fig. 8c-e). In the caeliferan orthopterans, the proximal scoloparium was thought to be lost in the hind leg FeCO, since only one cluster of neurons had been described (Usherwood et al., 1968; Zill, 1985a). However, a distal group of tiny sensory neurons in the metathoracic FeCO of the locust are supplied by a separate nerve which overlays the FeCO and joins its sensory nerve proximally (Matheson and Field, 1990). Although the group appears to be an integral part of the main FeCO, its innervation by a separate nerve, and composition of tiny neurons were taken as evidence for serial homology to the proximal scoloparium (which contains tiny neurons) in the fore- and middle legs. Further support for homology comes from developmental evidence in the grasshopper Melanoplus. The FeCO of all three leg pairs arises as a single invagination and subsequent proliferation of an undifferentiated cell mass in the femur. At day 23, the mushroom-shaped cell mass of the fore- and middle legs splits off a distal portion and thereby forms the two separate scoloparia. In the hind leg, the cell mass does not split off a separate portion, but it differentiates into the large and small neurons of the adult FeCO (Slifer, 1935). Thus the tiny neurons in the FeCOs of all three legs have a common origin.

The advantages underlying the evolution of two scoloparia (three in dipteran FeCO: Lakes-Harlan and Pollack, 1993; see Section 3.5.3) and the
modification of them in the hind leg are essentially unexplored. In the middle leg, the proximal scoloparium is much more sensitive to vibration than the distal scoloparium, and only the distal scoloparium forms inputs to postural joint reflex circuits (Field and Pflüger, 1989). The implications are that the two scoloparia are functionally different and may have evolved from an ancestral proprioceptor organ sensitive to both vibratory and gross joint movements, in a similar fashion to the scheme proposed by Shaw (1994a) for the evolution of vibration and hearing sensitivity in insects (see Section 12.4.2).

12.4.2 Complex tibial organs

The concept of serial homology between the complex tibial organs (subgenual organ, crista acustica, intermediate organ) of orthopteran legs was based upon histological study of adult ensiferan organs (Friedrich, 1927, 1928). Later authors echoed the concept (Debaisieux, 1938), but clear proof of serial homology was not available until developmental studies showed that the complex tibial organs are derived from the same embryological tissue (anlage) in every leg (Meier and Reichert, 1990). Histological and physiological examination of the complex tibial organs within each leg revealed that the subgenual and intermediate organs develop similarly, but the crista acustica is greatly modified in the foreleg to accommodate sound reception (Rössler, 1992a,b). Cytological differences in morphology and attachment of the prothoracic scolopidia, and the obvious cuticular and tracheal modifications for the ear (such as the tympana), must represent modifications of the ancestral ground plan (Kutsch and Breidbach, 1994).

The physiology of the original ensiferan hearing system probably was adapted to low-frequency sound and vibration detection, and was common to all legs (Rössler, 1992a). Modifications of the foreleg led to an enhanced hearing system evolved to detect high-frequency sounds. Concomitant evolution of sound production and species-specific communication signals would have heightened selective pressure toward conspecifically-tuned sensitivity of the crista acustica in the forelegs.

Further insight into the evolution of hearing in insects was provided by the discovery that the subgenual organ of cockroaches is sensitive to airborne sound, as well as being exquisitely sensitive to substrate vibration (Shaw, 1994a). A potential route of sound propagation occurs through internal coupling of the SGO to a compressible tracheal compartment. Such coupling would enhance SGO sensitivity to vibratory stimuli propagating in the leg haemolymph, but the same coupling would preadapt the SGO for detection of airborne sound via spiracular openings of the tracheae. By contrast, the SGO of the termite Zootermopsis angusticollis is evidently suspended in haemolymph without coupling to a compressible component (Howse, 1968), and the system is comparatively insensitive, since little differential displacement of the SGO would occur in the incompressible haemolymph (Shaw,
Based upon these observations, Shaw proposed a scheme for the evolution of tibial hearing and vibration detectors in insects (Fig. 35). The ancestral state is represented by that still found in some termite species, where the SGO is a low efficiency vibration detector suspended in the haemolymph across the tibia (Fig. 35a). By coupling the SGO to a compressible tracheal sac (Fig. 35b) the vibrational sensitivity is increased, and sound detection is added to the system through airborne sound entering the tracheae, as found in extant cockroach species. Finally, specialised scolopidia split off from the SGO and became intimately associated with a highly modified tracheal sac coupled to one or two thin tympanal membranes, to give rise to an enhanced hearing system (Fig. 35c). A further consequence of this modification, and a requirement for good hearing, was mechanical isolation of the hearing organ (crista acustica) from vibratory stimuli in the haemolymph. This system is represented in the ensiferan tibial hearing organs. Although not stated in Shaw’s scheme, it is likely that the tracheal organs (crista acusticae) in the atympanate middle and hind legs of many insect species represents an intermediate stage in which a row of scolopidia have become separated from
the SGO but are not coupled to tympanal ear structures. It has been proposed that such tracheal organs served as proprioceptors for haemolymph pressure detection (Debaisieux, 1938). In one atympanate tettigoniid species, *Phasmosodes ranatriziformes*, the full complement of complex tibial organs is present in the forelegs, but the insect is deaf to airborne sound, and may represent the above condition (Lakes-Harlan *et al.* 1991).

12.5 **HOMOLOGY OF SCOLOPIDIA AND CUTICULAR SENSILLA**

Early histologists such as Berlese (1909) and Demoll (1917) recognised the close similarities between scolopidia, hair sensilla and campaniform sensilla, and first suggested that they are all homologous (Schmidt, 1969, 1973; Moulins, 1976). Later, Thurm (1965) extended the comparison to the level of stimulus reception and transduction. The cuticular sensilla have a common structural plan based upon three (usually) support cells wrapping concentrically around the ciliated outer dendritic segment (Zacharuk, 1985). Each cell has received various names (Table 7), some of which imply developmental roles in the formation of the sensillum. Invariably, the inner cell forms the dendritic sheath, the median cell forms the hair or bristle, and the outer cell forms the socket.

A summary in support of homology of these cells to the scolopidial cells, based upon ultrastructural evidence, was given by Schmidt (1973) as follows:

1. Sensory hairs and scolopidia are innervated by neurons whose dendrites bearing a sensory cilium.

2. The satellite cells of hair sensilla, campaniform sensilla, amphinematic sensilla and some mononematic sensilla are usually arranged similarly.

3. The scolopale is formed by the innermost sheath cell in scolopidia, as is the case for the cuticular sheath of hair and campaniform sensilla.

4. The moulting process is the same in hair sensilla, campaniform sensilla and amphinematic scolopidia. The cuticular sheaths, and the scolopale of amphinematic scolopidia, are shed with the old cuticle during ecdysis. The somewhat different moulting mode of mononematic scolopidia can be explained by the lack of a cuticular connection between scolopidium and cuticle.

Schmidt concluded that the scolopale and cuticular dendritic sheath are homologous structures, and that the scolopale cell is homologous with the *theogen cell* of hair and campaniform sensilla. The cap (attachment) cell is homologous to the *trichogen cell*, and the accessory cell is homologous to the *tormogen cell* (summarised in Table 7).

Since Schmidt's work, genetic and developmental studies have provided further support for these homologies, with one exception. Genetic evidence comes from the *cut* locus in *Drosophila*, where a single gene mutation can transform hair sensilla into chordotonal sensilla (Bodmer *et al.* 1987).
Table 7. Nomenclature and structures formed by cells of scolopidia and hair sensilla. Homologies between cells of the two types of sensillum are based upon Schmidt (1973).

<table>
<thead>
<tr>
<th>Cell</th>
<th>Scolopidium Structure formed</th>
<th>Cell</th>
<th>Hair sensillum Structure formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scolopale cell¹</td>
<td>Scolopale</td>
<td>Thecogen cell³</td>
<td>Sheath surrounding ciliated dendrite</td>
</tr>
<tr>
<td>Stiftzelle²</td>
<td></td>
<td>Neurilemma cell³</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inner sheath cell³</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neurilemmzelle⁴</td>
<td></td>
</tr>
<tr>
<td>Attachment cell¹</td>
<td>Cap or tube</td>
<td>Trichogen cell³</td>
<td>Cuticular hair or bristle</td>
</tr>
<tr>
<td>Cap cell</td>
<td></td>
<td>Median sheath cell⁵</td>
<td></td>
</tr>
<tr>
<td>Kappenzelle²</td>
<td></td>
<td>Intermediate sheath cell³</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trichogene Zelle⁴</td>
<td></td>
</tr>
<tr>
<td>Accessory cell¹</td>
<td>Connection to cuticle</td>
<td>Tormogen cell³</td>
<td>Cuticular socket</td>
</tr>
<tr>
<td>(if present)</td>
<td></td>
<td>Tormogene Zelle⁴</td>
<td></td>
</tr>
<tr>
<td>Akzessorische Zelle²</td>
<td></td>
<td>Outer sheath cell³</td>
<td></td>
</tr>
<tr>
<td>Sensory neuron¹</td>
<td>Ciliated dendritic segment</td>
<td>Sensory neuron¹</td>
<td>Ciliated outer dendritic segment</td>
</tr>
<tr>
<td>Sinneszelle²</td>
<td></td>
<td>Sinneszelle⁴</td>
<td></td>
</tr>
</tbody>
</table>

The table is modified from Schmidt (1973) and terminology is compiled from the following sources: ¹Moulins (1976); ²Jägers-Rohr (1968); ³Zacharuk (1985); ⁴Lawrence (1966); ⁵Schmidt (1973)
Developmental studies of the two sensilla types show that each can arise from the same precursor cell (sensory mother cell), as shown in Fig. 30, depending upon the state of the *cut* gene “switch”. However the downstream cascade that follows *cut* action as a type-selector gene (Table 6) results in a slightly different lineage for the component cells of each type of sensillum (Fig. 30a,c), resulting ultimately in an exception to Schmidt’s (1973) scheme In both lineages, one daughter cell from the first division always produces the neuron and the innermost support cell (scolopale cell, thecogen cell), initially supporting the homology. The other daughter cell from the first division always produces the intermediate support cell (accessory cell, tormogen cell), again supporting the homology. However the third pair of proposed cell homologues, the attachment cell and the trichogen cell, arise from non-shared daughter cell precursors, which does not support the homology. This is a significant difference since these two cells produce clearly-recognisable extracellular structures, the cap and the hair, which have equivalent functions (both serve as distal anchor points of the dendritic outer segment). Furthermore, within the two lineages, a third cell division is required to achieve the final four cells of a scolopidium, while only two rounds of division lead to the four cells of a cuticular sensillum. Thus it becomes problematic to describe the homology of component cells in the two sensilla at the level of their genetic lineage.

### 12.6 ORIGIN AND EVOLUTION OF SCOLOPIDIA

A number of authors used the earlier cell homology arguments to propose that insect chordotonal organs were derived evolutionarily from cuticular hair sensilla (e.g. Horridge, 1965; Schmidt, 1969). Reduction of the ancestral (plesiomorphic) hair shaft would lead to the morphology of a campaniform sensillum, and migration of the sensillum below the cuticle could have produced a scolopidium. Alternatively, Kouyama and Shimozawa (1982), and Schmidt and Gnatzy (1984), proposed that the scolopidium is the primitive form of mechanoreceptor in arthropods, whether cuticular or subcuticular. Hair sensilla and campaniform sensilla would therefore represent derived conditions. This conclusion was based upon two key observations: a) many mechanoreceptors, including mechanoreceptor hair sensilla in crustaceans and myriapods, have scolopale-like structures surrounding distal ciliated segments of sensory neurons (reviewed by Kouyama and Shimozawa, 1982; Zacharuk, 1985; Crouau, 1995); and b) in general, the cell assemblages of the sensilla in cuticular and chordotonal receptors appear homologous in the various arthropods. The discovery of solitary scolopidia distributed in the genital chamber integument of the cricket has led to the suggestion of another scheme (Sugawara, 1996). In this case the original condition is thought to be single scolopidia with Type 1 dendrites embedded in soft cuticle, and no developed cuticular structures. The Type 1 cilium most closely resembles that of motile cilia, although the central pair of microtubules of the axoneme is absent. Loss
of the axoneme and an increase in microtubule density in the cilium (seen in Type 2 scolopidia embedded in the genital chamber cuticle) would lead to the tubular body that is characteristic of chemo- and mechanoreceptive hair sensilla and campaniform sensilla. This would be accompanied by development of associated cuticular structures. In the cricket genital Type 2 scolopidia, the cilium is bifurcated apically. This is thought to foreshadow the dendritic ramification seen in chemoreceptor neurons, which penetrate multiple pores of a hair shaft (Zacharuk, 1985). Chordotonal organs are thought to have developed as scolopidia withdrew the into the body cavity (and presumably associated into clusters as scoloparia linked to the cuticle by elaborations of attachment cell connections).

Collembolans were thought to offer a unique opportunity to approach the problem of scolopidial origin, since their gross structure appears unchanged since the Devonian. The collembolan antenna has chemosensory pegs, each containing not only chemosensory innervation but also a separate scolopidium attached to the base. This suggested a transition condition with incomplete internalisation of the scolopidium (Altner and Theis, 1984). Although the collembolan scolopidial sensory peg is certainly an intermediate form, it can be fitted into any of the evolutionary scenarios above and does not solve the basic problem of derivation. Since extant arthropod species present a mosaic of primitive and advanced characters, the question of evolutionary derivation is complex. It would be useful to look at other phylogenetically-related groups such as Onychophora and Annelida for evidence of plesiomorphy in ciliated sensilla, and to determine whether scolopidial structures are indeed unique to arthropods.

Within the insects, chordotonal organs have undergone evolutionary changes in the cellular composition of scolopidia. In Johnston’s organ of some insect orders, one of the four progeny of the sensory mother cell (Fig. 30) for amphinematic scolopidia has been lost during evolution. Normally there are three enveloping cells around an amphinematic scolopidium (the scolopale cell, attachment cell, and accessory cell, Table 7 and Fig. 11b), but in the holometabolous insects (Lepidoptera, Mecoptera, Diptera) examined by Schmidt (1973), the outer cell (= accessory cell) is lacking. The same condition, with only two enveloping cells, has been found in amphinematic scolopidia of several phylogenetically distant hemimetabolous insects (Blattaria, Isoptera, Hemiptera) as well as the ametabolous Thysanoptera (Toh, 1981; Howse, 1968; Howse and Claridge, 1970; Bode, 1986). It is likely that the reduction in cell number evolved independently in these diverse groups.

In mononematic scolopidia the most frequent condition is two enveloping cells in both hemimetabolous and holometabolous insects, although Bode (1986) pointed out that the plesiomorphic condition of three cells is found in some Orthoptera and Coleoptera. The loss of the accessory cell undoubtedly is associated with the nature of attachment of the chordotonal organ to the integument. The attachment cell is usually subepidermal (and may be
incorporated in a connective tissue strand) and attaches to the epidermal accessory cell in
tympanal organs, subgenual organs and connective chordotonal organs. However in some other
chordotonal organs with mononematic scolopidia, such as the antennal connective chordotonal
organ, the accessory cell may be lost and the attachment cell may connect directly to the cuticle
(Moulins, 1976).

The significance of the reduction of enveloping cells in a scolopidium is not clear, but is open
to experimental analysis. For example, histological differences between direct and indirect
connection of the attachment cell to the cuticle may bear upon biophysical (e.g. elastic)
properties of the connection, and therefore relate to physiological sensitivity or specificity of
chordotonal organs.

13. Conclusions

In the Introduction we stressed that current research on chordotonal organs is no longer
confined to sensory neurophysiologists, but is now undertaken by a diverse assemblage of
investigators in many areas of biology. It was our intention to incorporate into this synthesis
comments on critical and potentially productive areas for research, and to thus generate further
progress by workers from a variety of disciplines. We hope that by encouraging a multi-
disciplinary approach, research on chordotonal organs will overcome some of the obstacles that
have hindered past progress. For example, there is still a need to combine the technologies of
neurophysiology with those of genetics, electron microscopy, membrane biochemistry and
biophysics to further elucidate the mechanisms that endow chordotonal sensilla with their
different physiological properties.

Of the research questions raised in the Introduction, the foremost was the longstanding mystery
of how mechanical stimuli are applied to the neuronal dendritic outer segment. This remains
unsolved, although many hypotheses about transduction have been proposed (Sections 5.2, 6).
Initial progress has been made on how the receptor potential is conveyed to the dendritic inner
segment and thence to the trigger zone (Sections 6.2, 6.3), but this needs to be established for
more than one type of scolopidium. The presence and function of mechanically-activated ion
conductance channels have been touched upon but there is still no evidence for their existence
in the dendritic region of scolopidial neurons (Section 6.1).

Our understanding of the elaborate ultrastructural components of scolopidia has been enhanced
by immunochemical evidence for potentially contractile proteins in certain structures. Although
the evidence is suggestive, crucial experimental demonstration of their roles is lacking
(Sections 4.3.3, 4.4). The need for investigation of the ultrastructural basis of different
physiological responses in scolopidia (e.g. tonic versus phasic, acceleration-
sensitivity *versus* velocity sensitivity) was raised earlier (Moulins, 1976; McIver, 1985) and still remains unapproached by researchers (Section 7.4.2). The contributions of various mechanical components to the different physiological responses of chordotonal organs is another area requiring research. Progress on this question has been made for tympanal organs (Section 7.4.2.4) but not for joint chordotonal organs.

We now have a greatly improved knowledge of both the central projections of chordotonal organs, and the neural circuits that carry and process their information (Sections 8, 9). Nevertheless, there are still major questions to be answered in these areas. The central projections of auditory chordotonal organ afferents are organised tonotopically within the auditory neuropil, but little is known of the patterns underlying the organisation of afferents from other types of chordotonal afferents. In at least one instance in insects, aspects of central branching pattern correlate with receptors’ different responses to movements of a leg joint in different positions. We know that not all afferent neurons from a chordotonal organ synapse with a particular motor neuron or interneuron, but the rules governing exactly which afferents connect with each target are poorly understood.

Ablation of proprioceptive chordotonal organs may have little effect on overt behaviour, or the effects may be quickly countered, yet we do not have a good understanding of what other sense organs have inputs to the relevant neural circuits, or how the various inputs are integrated. It would be valuable to investigate convergence of inputs from diverse sense organs onto central target neurons that are known to be important in mediating behaviours influenced by chordotonal organs.

Chordotonal organs are not known to receive direct efferent control, but the strengths of the reflexes that they mediate can vary greatly. Presynaptic inhibition of the sensory output terminals provides one mechanism underlying this variability, and the responses of the receptors can be directly affected by peripheral application of the neuromodulator octopamine. Even so, it seems likely that there is more specific local release of neuromodulators into the peripheral receptors. The possibility that neuromodulatory neurons have peripheral release sites on or near chordotonal organs should therefore be investigated in more detail.

Much new knowledge about chordotonal organs has come from the study of genetics and development. Many of the questions in these areas were not even envisaged in past reviews of chordotonal organs, while former questions about derivation and genetic control of scolopidia during development have been abundantly answered (Sections 10, 11, 12). The startling discovery that, during development, scolopidia can be transformed into hair sensilla and *vice versa* (Sections 11.1, 11.3) has reshaped our understanding of sense organ development in insects, and consolidated knowledge of evolution and homology of insect sensilla.

The roles of chordotonal organs in behaviour are now known to be manifold
and sophisticated. They are involved in proprioception, audition and detection of vibration (at least), and participate in behaviours ranging from simple resistance reflexes to walking and flying. Chordotonal organs range in complexity from receptors containing a single neuron to complex structures containing many hundreds of neurons. Their output targets can be motor neurons, local or intersegmental interneurons. The sensory neurons are recognisable early in development, and have proved amenable to genetic manipulation. All these attributes make insect chordotonal organs extremely valuable tools for furthering our understanding of the principles that underlie functioning of nervous systems.

14. Acknowledgements

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15. Abbreviations

15.1 SENSE ORGANS

aCO anterior CO of CO system
ajCO anterior joint CO spanning the thorax/coxa joint
apCO apodemal CO in thorax of locusts
ASO apical sense organ
cCO coxal CO of CO system
ch embryonic chordotonal organ in *Drosophila* (see es)
CO chordotonal organ
COS system of chordotonal organs (aCO, cCO, pCO, vCO) linked by ligaments in the thorax
dch3 cluster of 3 dorsal scolopidia in *Drosophila* embryo

dh1 dorsal (trichoid) hair sensory neuron in *Drosophila* embryo

es embryonic external sensillum in *Drosophila* (see cs)

FeCO femoral CO

lch5 cluster of 5 lateral scolopidia in *Drosophila* embryo

MRO muscle receptor organ (receptor neuron and associated muscle)

myoCO myochordotonal organ in thorax

pCO posterior CO of CO system

pjCO posterior joint CO spanning the thorax/coxa joint

plCO pleural CO of locust abdomen

SGO subgenual organ (vibration receptor) in leg

SR strand receptor (receptor neuron with central soma)

vch1 ventral scolopidium in *Drosophila* embryo

vCO ventral CO of CO system

15.2 **GANGLIONIC REGIONS AND CELL TYPES**

aISN anterior intermediate sensory neuropil (=aRT, mVAC)

aLAC anterior LAC

aRT anterior ring tract (=aISN, mVAC)

aVAC anterior VAC

AVC anterior ventral commissure

cdl caudo-dorso-lateral terminal region of stick insect ganglion

CNS central nervous system

DCI-VI dorsal commissures I-VI

DIT dorsal intermediate tract

dVCLII dorsal part of ventral commissural loop II

Fe1 femoral guidepost cell in developing leg

FETi fast extensor tibiae motor neuron

LAC lateral association centre

lVAC lateral VAC

mVAC medial VAC (=aRT, aISN)

MVT median ventral tract

pLAC posterior LAC

PNS peripheral nervous system

rdl rostro-dorso-lateral terminal region of stick insect ganglion

SETi slow extensor tibiae motor neuron

SMC supra median commissure or sensory mother cell (also SOP)

SOP sense organ precursor cell (also SMC sense 2)

Ta1 tarsal pioneer neuron

T1i tibial pioneer neuron

Tr1 trochanteral guidepost cell in developing leg

VAC ventral association centre

VIT ventral intermediate tract
### 15.3 Others

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>bp</td>
<td>base-pairs (see kb)</td>
</tr>
<tr>
<td>CF</td>
<td>characteristic frequency (of sound evoking peak response)</td>
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<tr>
<td>DiI</td>
<td>1,1’,dioctadecyl-3,3,3’3’-tetramethylindocarbocyanine perchlorate (fluorescent dye)</td>
</tr>
<tr>
<td>ECM</td>
<td>extracellular matrix</td>
</tr>
<tr>
<td>EGF</td>
<td>epidermal growth factor</td>
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<tr>
<td>EPSP</td>
<td>excitatory postsynaptic potential</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-aminobutyric acid (neurotransmitter)</td>
</tr>
<tr>
<td>HLH</td>
<td>helix-loop-helix (protein structure)</td>
</tr>
<tr>
<td>HRP</td>
<td>horseradish peroxidase (electron dense dye and immunolabel)</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine (neurotransmitter)</td>
</tr>
<tr>
<td>IPSP</td>
<td>inhibitory postsynaptic potential</td>
</tr>
<tr>
<td>kb</td>
<td>kilo base-pairs (see bp)</td>
</tr>
<tr>
<td>kDa</td>
<td>kilo Daltons</td>
</tr>
<tr>
<td>MAC</td>
<td>mechanically activated channel</td>
</tr>
<tr>
<td>MAP</td>
<td>microtubule associated protein</td>
</tr>
<tr>
<td>MIC</td>
<td>membrane integrated cone</td>
</tr>
<tr>
<td>PSP</td>
<td>postsynaptic potential</td>
</tr>
<tr>
<td>RSA</td>
<td>rectifying stretch activated (channel)</td>
</tr>
<tr>
<td>SA</td>
<td>stretch activated (channel)</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscope</td>
</tr>
<tr>
<td>VEP</td>
<td>vibration evoked potential</td>
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### 16. References


Blochlinger, K., Bodmer, R., Jan, L. Y. and Jan, Y. N. (1990). Patterns of expression of
Cut, a protein required for external sensory organ development in wild-type and cut mutant Drosophila embryos. Genes Dev. 4: 1332-1331.


Collin, S. P. (1985). The central morphology of mechanoreceptor afferents in the


Orona, E. and Agee, H. R. (1987). Thoracic mechanoreceptors in the wing bases of
Heliothis zea (Lepidoptera: Noctuidae) and their central projections. *J. Insect Physiol.* 33: 713-721.


Yack, J. E. (1992). A multiterminal stretch receptor, chordotonal organ, and hair plate at the wing-hinge of Manduca sexta: unravelling the mystery of the noctuid moth ear.


Plate 1. Demonstration of actin, actinin and centrin-like reactivity in Johnston’s organ scolopidia of the cockroach *Periplaneta*, using immunolabelling. Mononematic scolopidia are shown in confocal laser scanning reconstructions (a, c) and Nomarski interference optical micrographs of the same preparations, respectively (b,d). a. Indirect immunofluorescence of anti-α-actinin (red, rhodamine) reveals α-actinin in the ciliary rootlets of the inner dendritic segment of a sensory neuron (arrowheads in a and b). Phalloidin-FITC (green) labels actin in the scolopale below the cap (arrow in b). c. Indirect immunofluorescence of anti-centrin (green, FITC) and phalloidin-rhodamine (red). The phalloidin-rhodamine labels actin filament bundles below the scolopodial caps (arrow in d) and FITC fluorescence labels centrin in the ciliary roots (arrowheads). Scale: 10 µm. a-d, Wolfrum (1991a), with permission.
Plate 2. Transformation of cuticular hair sensilla into scolpidia by the cut gene in Drosophila. Transformation revealed by double staining the lch5 cluster of scolpidia in 14h wild-type and cut⁴¹⁵ embryos with the antibodies 49C4 and 21A6 (a,b) and of wild-type and cut⁴¹¹ adult sensilla with antibodies 21A6 and 58C12 (c,d). Open arrows indicate normal scolpales; curved open arrows indicate normal wild-type chordotonal neurons; asterisk indicates ⁵ᵗʰ scolopale of the cluster, whose neuron did not stain with 49C4 in normal embryo. a. Wild-type lch5 scolpidea and normal terminal “dot” seen at the tip of a cuticular sensillum neuron (arrowhead). b. Mutant embryo in which cuticular sensillum dots are transformed into scolpales (short solid arrows) with neurons (solid curved arrows) now staining with 49C4. The positions and orientations of the transformed hair sensilla are as expected for wild-type organs (see Fig. 24d). c. Adult wild-type cuticular bristle showing dot at tip of sensory neuron dendrite which inserts into base of bristle. d. Adult cut⁴¹¹ mutant bristle clearly showing transformed scolopale and neuron with elongated dendrite beneath socket. Scale: a,b, 50µm; c,d, 12 µm. a,b, Bodmer et al. (1987), with permission; c,d, R. Bodmer (unpublished), with permission.