Comp. Biochem. Physiol. Vol. 82A, No. 3, pp. 525–529, 1985
Printed in Great Britain

0300-9629/85 $3.00 + 0.00

CUTICULAR HAIR ORGANS EVOKING REFLEXIVE CLOSING AND OPENING OF THE CRAYFISH CLAW

BRIAN J. NORRIS* and H. BERNARD HARTMAN†
Department of Biological Sciences, Texas Tech. University, Lubbock, TX 79409, USA.
Telephone: (806) 742-2715

(*Received* 14 *February* 1985)

Abstract—1. Cuticular hair organs on the index of the propus of the chelae of the crayfish *Procambarus simulans simulans* Faxon evoke reflexive closing and opening of the dactyl when stimulated by touch.

2. These receptor organs are arranged in eight linear fields (A–H), with each row innervated by a separate branch of an afferent nerve.

3. Nerve branches A and F innervate receptors on either side of the cutting edge of the propus; these organs trigger claw closing. Branches B and G innervate receptors driving both claw opening and closing; branches C, D, E and H innervate hair organs that drive claw opening.

4. The hair organs, which consist of groups of simple or pappose setae, can be divided into four types based on morphology.

5. The cuticular hair organs show directional sensitivity.

INTRODUCTION

One of the best understood invertebrate neuromuscular systems is that which controls the claws and walking legs of decapod Crustacea, yet very little is known about how other neurons interact with this system to produce even simple behaviors. The firing of chordotonal organs during passive movements of the limb and the effect of activity in chordotonal organs on the activity of related muscles has been well characterized, but the central connectivity mediating reflexes is still unknown. Postural reflexes have been particularly difficult to study because the associated change in the rate of firing of the motor neurons is small, and statistical techniques are necessary to show that a change has occurred (for a recent review see Wiens, 1982).

Touching a crayfish along the inside border of the opened claw elicits a reflexive closing, while a touch elsewhere on the claw triggers opening (von Uxkühl and Gross, 1913; Barnes, 1932). Von Uxkühl and Gross (1913) showed that both reflex arcs are isolated to the cheliped ganglion. We have found that receptors mediating these reflexes are cuticular hair organs that are easily manipulated. They are located on the index of the propodite and the dactylopodite where no muscles are found, leaving 1–2 cm of purely afferent nerve available. Also, these reflexes are simple, stereotyped behaviors which exhibit the simple learning (habituation, dishabituation, etc.) seen in other invertebrate preparations (Wiens, 1982).

The aim of this study was to examine the morphology, physiology and distribution of the cuticular hair organs on the index of the propodite which mediate opening and closing reflexes of the dactyl in the crayfish *Procambarus simulans simulans* Faxon.

MATERIALS AND METHODS

Crayfish (*Procambarus simulans simulans* Faxon) were collected from a small spring-fed pond one mile south of Bull Lake, near Littlefield, Texas. The animals were kept in aquaria containing fresh water at room temperature (21°C) and fed beef liver once a week. Animals were chosen for experimentation regardless of sex.

Two experimental preparations were used. The first was a whole animal preparation used to record the claw closing reflex. The dorsal surface of the carapace of the animal was fixed to a lucite rod with epoxy cement. Two enamel coated pairs of silver wires were attached to the right cheliped. For recording afferent activity one pair of wires, the tips scraped clean of enamel, was inserted into the base of the index of the propodite so as to span the afferent nerves. The other pair was inserted through tiny holes in the exoskeleton into the closer muscle to record muscle activity. The detected signals were processed by conventional methods.

The animal was positioned with the walking legs and the abdomen in contact with the substrate. Claw dactyl reflexes were triggered by touching the propodite with a pipe cleaner which was trimmed to 3 mm diameter. The animal had to be in an aroused state for reflexes to occur.

In the second preparation, the cutter claw was removed at the carpopodite–propodite joint, and bathed in van Harreveld's saline (van Harreveld, 1936), buffered with TRIZMA at pH 7.4 and maintained at 16°C. Windows ~4 × 4 mm were cut on the ventral and dorsal sides of the propodite and part of the closer muscle was removed to reveal the afferent nerves. Individual nerve branches were drawn into suction electrodes for recording action potentials. Cuticular hair organs were stimulated by a small wooden dowel sharpened to a point, a stream of saline delivered by pipette was also used as a stimulus. For purposes of mapping, a photographic slide of the claw index was projected onto a sheet of paper inside the recording cage, where the position and response of each hair organ could be noted. After the recording session was complete, the claw index was stained in methylene blue to highlight the hair organs. For detailed study, several claws were coated

*Present address: Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138, USA.
†Person to whom reprint requests should be addressed.

Fig. 1. Electrical activity in the claw closer muscle and afferent nerves in the propus index during reflexive closing. Top trace: myogram from closer muscle; bottom trace: afferent nerve activity. Prior to touch stimulation (arrow) potentials resulting from closer inhibitory neuron activity are evident in the myogram. When the propus is touched, a burst of action potentials in the afferent nerve is followed by a reflexive train of excitatory potentials in the muscle. Time bar: 200 msec.

with gold–palladium and scanning electron micrographs were made of the entire index and individual hair organs.

RESULTS

Claw closing reflex

We have observed that when the aroused but tethered crayfish is touched with an object near the edge of the dactyl or propus of a cheliped, the dactyl closes by reflex action, apparently in an effort to grasp the object. If the same stimulus is now applied to more peripheral areas of the claw, the dactyl opens. An extracellular recording from the afferent bundle in the propodite and a myogram record from the closer muscle to closing evoked by touch is seen in Fig. 1. In the myogram, the closer inhibitory neuron (CI) can be seen firing, prior to stimulation. Following touch, a burst of action potentials in the afferent nerve was followed shortly afterwards by an excitatory reflexive response in the closer myogram. The average reflexive delay time between the afferent burst and the closer muscle response varied from 30 to 80 msec for 10 trials in one carefully measured preparation. The average delay time was 60 msec. Fast closer excitatory neuron (FCE) responses could not be distinguished from those of the slower closer excitatory neuron (CE) in the myograms, but behaviorally it was apparent that the initial response was by the FCE. No records were made from the opener muscle.

Nerve branching patterns and fields of innervation

In order to locate the cuticular hair organs responsible for evoking the claw closing reflex, the afferent nerves were exposed in the propodite. A characteristic branching pattern was observed. At the carpopodite–propodite joint, the afferent bundle divides into two nerves; one innervating the medial face of the index and other the lateral side. As seen in Fig. 2, that bundle projecting to the ventral face subdivides into five branches (A–E), while that innervating the dorsal side subdivides into three (F–H). This pattern was conserved over the seven preparations examined.

Mechanically stimulating individual hair organs, while recording from nerve branches, revealed that each evoked a response in only one branch. Furthermore, in the seven preparations examined, a sense organ in a specific location always elicited a response in the same branch. It was also found that the branching pattern corresponded to fields of innervation (Fig. 2A, C). On the ventral face of the index, four linear fields (A–D) of hair organs are seen to project distally, while one field (E) is situated proximally. Hair organs forming three linear fields (F–H) serve the dorsal face. In an roused crayfish, the touch of a single hair organ in fields A or F triggers claw closing. Touch of receptors in fields B and G triggers closing and opening; the hair organs located distally in those fields stimulate closing. Claw opening is triggered by receptors in fields C, D, E and H (Fig. 2A, C).

Hair organ anatomy and distribution

Scanning electron micrographs were taken of the index of the propus to determine the morphology and distribution of the mechanoreceptors. Hair organs were found to be composed of either simple setae, or a combination of simple and pappose setae (setae types from Factor, 1978). Lengths of setae from claws stained with methylene blue were measured under a dissecting scope. The pappose setae are all approximately the same length (*ca* 200 μm), while the length of the simple setae varies from one receptor type to another.

Based on electron micrographs, these cuticular hair organs can be divided into four types (Fig. 3A–D). Type 1 receptors consist of a clump of 7–20 long, simple setae (200 μm), bordered by 5–7 pappose setae

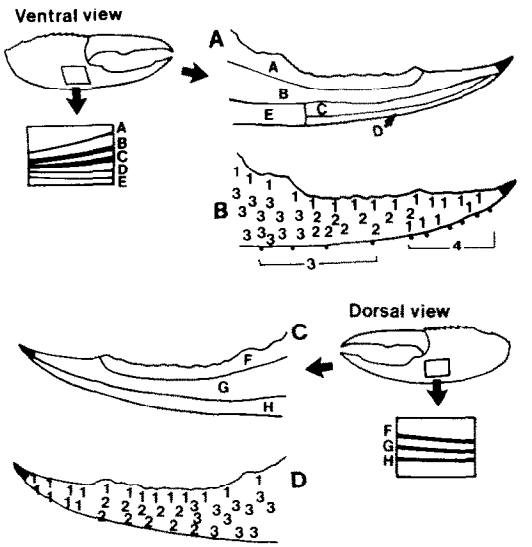

Fig. 2. Ventral and dorsal views of the crayfish claw showing the branching pattern of the afferent nerve innervating the propus index, the fields of innervation (A, C), and the location of each of the four types of cuticular hair organs (B, D).

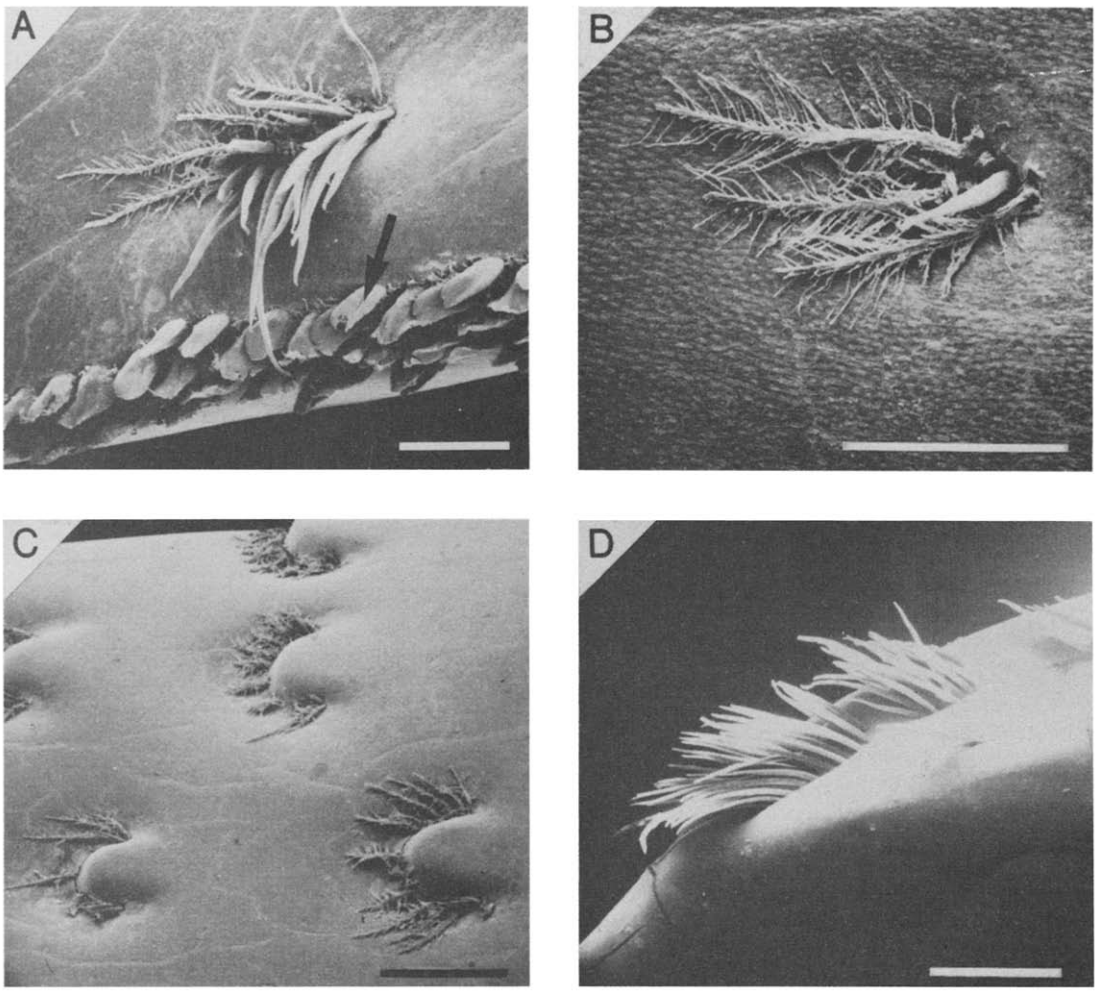

Fig. 3. Scanning electron micrographs of the four mechanoreceptor types found on the index of the propus. A through D are Types 1 through 4 respectively. The arrow in A points to one of the blade-shaped structures assumed to be chemoreceptive. Scale bar: 200 μm (A, B) and 500 μm (C, D).

(Fig. 3A). Type 2 receptors are similar to Type 1 receptors, but have only 1–3 short simple setae (80 μm), and 3–5 pappose setae (Fig. 3B). Type 3 receptors are fan-shaped arrays of pappose setae bordered by 1 or 2 short simple setae (60 μm). The Type 3 receptors form either a complete fan, or have a gap in the center (Fig. 3C). Type 4 receptors consist of arrays of 10–50 very long (200–600 μm) simple setae (Fig. 3D). In addition, the cutting edge of the propus is lined with blade-like structures, each measuring $\sim 40 \times 100$ μm (Fig. 3A). These do not respond to the touch stimulus.

The distribution of the four types of cuticular hair organs can be noted in Figs 2B and D. While fields A and F are populated by Type 1 receptors only, the other fields are composed of two or three types of hair organs. Fields B, G and H consist of Type 1 receptors distally, with Types 2 and 3 positioned proximally. Types 1 and 2 receptors are found in field C. The distal portion of field D contains several Type 4 receptors, with two Type 3 receptors proximally. it is possible to subdivide a nerve such as B into three branches, one innervating Type 1 receptors, another Type 2 receptors and a third Type 3 receptors. Thus, the major nerve branching pattern corresponds to receptor rows along the index, but only fields A and F contain one type of hair organ.

Hair organ innervation and directionality

Recordings made from the afferent nerves revealed that each receptor type had a somewhat characteristic output and directional sensitivity. In these experiments a nerve was drawn into a suction electrode and the appropriate hair organs were stimulated. It should be noted that while the setae of each hair organ are individually articulated, no effort was made to stimulate single hairs. Rather, a probe or jet of saline from a pipette to the entire hair organ was employed. When stimulated, Type I receptors responded with 1 or 2 units (distinct amplitude action potentials) and had the largest spikes and therefore the largest axons of all receptor types (Fig. 4A). These receptors also exhibited the lowest thresholds responding to very slight wave motions and disturbances. Indeed, in many preparations it was necessary to destroy the most distal Type 1 hair organs in order to observe the neurophysiological responses of the more proximal receptors in the field. Type 2 hair

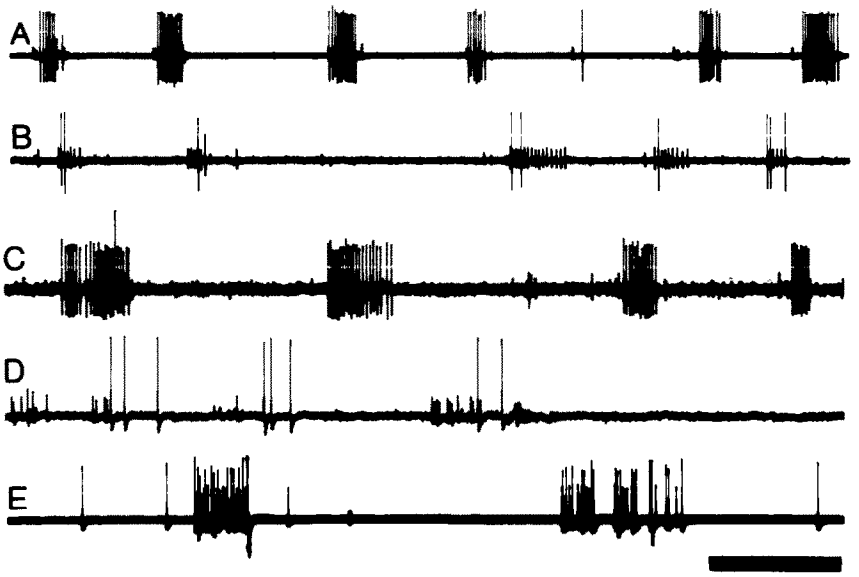

Fig. 4. Recordings of action potentials illustrating the responses of Type 1 through Type 4 cuticular hair organs (A, B, C, D) to touch stimulation; the action potentials in E are responses to pressure applied to the epicuticular cap. Scale bar: 100 msec.

organs (Fig. 4B) responded with one or two small units and occasionally a single large unit. Type 3 hair organs (Fig. 4C) drove either one or two small units, and if a Type 3 receptor drove two units, one unit was driven by the medial setae and the other by the lateral ones. One to four medium to small-sized units were excited by stimulation of the Type 4 hair organs (Fig. 4D). Pressure applied by a sharpened wooden dowel to the epicuticular cap evoked an output by three or four very large units in branch B (Fig. 4E). The variation in response by units resulted from non-uniformity of the stimulus. It should be noted, however, that whenever it was possible to stimulate a single seta, based on action potential size, that hair was always singly innervated.

When testing hair organ response, the stimulus was applied from different directions and the total numbers of spikes were counted for each stimulation. Directionality was assigned on the basis of the greatest response to a particular directional stimulus. Even with the non-uniform stimulus it soon became evident that directionality could be predicted on the basis of setae orientation. The setae of Types 1 and 2 hair organs were usually pointed in one direction (see Fig. 3A, B). If these receptors showed any directionality it was for a stimulus that pushed the setae upright. Type 3 receptors also responded most strongly to stimuli that tended to right the setae; however, due to their fan-shaped morphology, they responded to broader directionality (*ca* 180°) of mechanical stimulation (Fig. 3C). In contrast, stimuli that flattened the setae against the cuticle excited the Type 4 hair organs. Type 1 receptors located along the inner edge of the claw are for the most part sensitive to downwardly directed stimuli. The Types 1 and 2 hair organs on the rest of the index are responsive to distal or proximal stimuli, while the Type 4 receptors respond to stimuli directed toward the claw tip. Although sensitive to a range of directional stimuli, Type 3 receptors were most affected by proximally directed movements.

DISCUSSION

We have found that cuticular hair organs on the index of the propus of the chelae of *P. simulans simulans* trigger reflexive opening and closing of the dactyl when mechanically stimulated. Those which evoke closing are Type 1 hair organs located specifically along the ventral and dorsal cutting edge of the propus. The axons from these receptors form nerve branches A and F. Nerve branches B and G contain axons of receptors which drive both opening and closing. Type 1 hair organs at the cutting edge of the propus drive closing, while Types 1, 2 and 3 receptors located more proximally in fields B and G drive dactyl opening. The remaining nerve branches (C, D, E and H) receive input from Types 1, 2, 3 and 4 hair organs. These trigger dactyl opening upon stimulation. Thus, afferent nerves driving closing only (A and F), or opening only (C, D, E and H) are easily isolated in this species.

While the row of blade-like structures along the inner edge of the index (Fig. 3E, F) would seem to be likely mechanoreceptor candidates, no preparation yielded afferent activity in any nerve to mechanical probing of these structures. Similar structures on the lobster *Homarus gammarus* (Shelton and Laverack, 1970) and hermit crabs (Fields, 1974) have been shown to be chemosensory. It seems apparent from our results that the adjacent Type 1 hair organs in the ventral and dorsal inner border of the propus index are responsible for triggering closing.

The orderly arrangement of receptors and their nerves is striking. Hair organ receptors in a particular row are innervated by the same nerve branch and tended to show similar directionality. This similarity of innervation and directionality between receptors in

the same row has only previously been reported in crustaceans in the telson of the crayfish *Procambarus clarkii* (Kennedy and Mellon, 1964; Kennedy, 1971; Wiese, 1976). While the telson receptor rows are not as orderly as those on the claw of *P. simulans simulans*, they are nonetheless arranged in elongated fields with each field innervated by a single nerve from the last abdominal ganglion. Wiese (1976) noted further that receptors within a row shared the same directional preference. Whilst the cuticular hair organs and setae of lobster mouthparts, pereiopods (Farmer, 1974; Factor, 1978) and claws (Solon and Cobb, 1980) are arranged in rows, no neurophysiologic studies examining the pattern of innervation have been reported.

Two types of setae, simple and pappose, are found within the same cuticular hair organ on the claw of this species. As both are mechanosensory it seems probable that they respond to different types of stimuli. If one assumes that the pappose setae, which have a large surface area, are motion detectors, then the simple filiform setae might be expected to respond to touch as a stimulus. Type 1 receptors positioned along the edge of the claw have numerous simple but also several pappose setae and would be expected to be both touch and motion sensitive. As the Type 2 receptors have a few simple setae compared to pappose setae, they would be expected to respond more readily to motion. The fan-like Type 3 receptors composed for the most part of pappose setae are ideally suited for motion detection. Moreover, the Type 4 hair organs, consisting of numerous simple setae, would be expected to be touch sensitive. Physiological recordings support this interpretation. It should also be noted that both Types 1 and 4 receptors are found along the narrow edges of the claw, while Types 2 and 3 cover the broad flattened areas.

The morphology and physiology of setae has been extensively studied in another arthropod appendage, the cerci of cockroaches and crickets. In both groups of insects, receptors of common morphology are arranged in rows which span many segments, each row is innervated by a single nerve, and the receptors in a row show nearly identical directional preference (Gnatzy and Schmidt, 1971, 1972; Edwards and Palka, 1974; Westin *et al.*, 1977; Dagan and Camhi, 1979; Hartman *et al.*, 1979). We suggest that it may be a common feature for mechanoreceptive setae and hair organs of arthropods to be arranged in rows, the rows to share the same nerve, and the receptors of a row to show the same directional preference.

Acknowledgement—The authors thank Becky A. Moulton for critical review of the manuscript.

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