CAMPANIFORM SENSILLA ON THE TACTILE SPINES OF THE LEGS OF THE COCKROACH*

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The mechanosensory structures associated with the cuticle of the legs of cockroaches include small tactile hairs arranged singly or in groups, groups of proprioceptive campaniform sensilla (Pringle, 1938) and large, prominent tactile spines of the femur and tibia. The characteristics of the afferent discharges from tactile spines, used in the present study, were originally described by Pumphrey (1936). The preparation is simple and fairly reproducible, each tactile spine giving rise to a slowly adapting single-unit discharge, and seems to offer promise as a useful sense organ in which to study mechanical transduction and encoding. Pringle & Wilson (1952) and Chapman & Smith (1963) have described adaptation in this receptor in terms of linear transfer functions. The nature of its adaptation is of inherent interest in sensory encoding, but it has not been possible to investigate or interpret this process meaningfully in terms of cellular mechanisms, because specific information about the structure of the sensory ending has been lacking.

Both the large tactile spines and the small sensory hairs of the cockroach are usually classified together as trichoid sensilla. While the structure of the sensory endings of tactile spines has apparently never been described, considerable histological work has been published on the small tactile hairs of many insect species; this is discussed in recent reviews by Slifer (1961) and Dethier (1963). The extensive camera lucida drawings of Hsu (1938) and the recent electron microscopic work of Slifer (1961) describe one structural arrangement commonly found in small tactile hairs. In this, the dendrite of a single bipolar neuron in the underlying epidermal layer is inserted into the cuticle at one edge of the hollow base of the hair, and mechanical excitation presumably results from deforming the dendritic surface when the hair is moved. A second type of arrangement has been proposed by Wolbarsht (1960), who has concluded from electrophysiological data from a number of species that the mechanosensitive terminal of the sensory neuron completely or almost completely occludes the basal region of the lumen of many hairs, since an appreciable receptor potential appears between the distal lumen and the tissues underlying the hair when the structure is excited. He suggested further that the annular ‘membrane’ seen at the base of the labial hairs of the blowfly in Larsen’s (1962) electron micrographs may in fact be part of the dendrite of the mechanosensory neuron.

In the present work, the failure to detect receptor potentials through the lumen of the much larger tactile spines of the cockroach led to a histological and electro-

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physiological search for the sensory structure. In this, it is shown that each tactile spine contains a single campaniform sensillum at its base, in the thick sclerotized cuticular wall of the exposed surface of the spine where it joins the soft cuticle of its socket. A preliminary report of this work has appeared (Chapman, 1963).

MATERIALS AND METHODS

All work was done with adult Periplaneta americana of various ages, ranging in weight from 0.8 to 1.7 g. The single tactile spine of the dorsal surface of the femur at the femoro-tibial joint of the meso- and metathoracic legs has been used extensively, but some observations have been made on representatives of all other tactile spines as well. On all femora these occur in two rows on the anterior and posterior margins of the ventral surface. The spines of the tibiae of all legs occur in five radially arranged longitudinal rows, the most distal members of which are usually offset somewhat with respect to the others. The numbers of spines increase progressively from the short prothoracic to the long metathoracic legs.

Morphology. For sectioned material, legs from freshly killed animals were fixed in the tetrahydrofuran—Eltringham fixative of Salthouse (1958), double embedded in Parloidin—paraffin, sectioned at 10 or 15 μ and examined under phase contrast. Methylene blue and Gairns's (1930) gold chloride staining were also attempted in some cases, but neither offered any particular advantage in visualizing nervous elements. Cleared cuticle preparations were made both from freshly killed specimens and from those which had first been studied electrophysiologically. Legs were digested in hot 1N-NaOH until they were translucent and no internal tissue was evident (5–10 min.); then they were passed through an ethanol series into cedarwood oil. Clearing of the cuticle progressed over a period of months, and 4–6 weeks was required to produce adequate transparency for examination and photomicrography.

Electrophysiology. Preparations were made essentially as described by Pringle & Wilson (1952). For electrophysiological work only the larger meso- and metathoracic legs were used. These were amputated through the trochanter under carbon dioxide anaesthesia; recovery of the donor generally occurred a few minutes thereafter. Femoral preparations were usually separated from the tibia to eliminate electrical activity from the tibia, but tibial preparations were usually studied without further dissection.

A small dab of petroleum jelly was usually applied to the cut ends to retard desiccation. Preparations not so treated lost as much as 10% of their weight per hour, while petroleum jelly reduced this by about half. It was often possible to record from preparations so treated for 5 hr. or more. In the preliminary attempts to record receptor potentials, using non-polarizable electrodes in Yeager's (1939) cockroach saline over one cut end of the leg, weight losses amounted to less than 1%.

Receptor-potential experiments. Leg preparations were usually mounted either by means of a pair of steel insect pins which could serve as recording electrodes for

* These positions are designated homologically. As the metathoracic leg of Periplaneta is normally carried, the dorsal spine of the femur lies in the horizontal plane and points forward. The dorsal spines of the tibia point laterally. Throughout this discussion the individual spines in a row have been designated numerically, counting from the proximal end of the row; the terms 'proximal' and 'distal' are used in the same sense as on the intact leg.
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afferent activity (Pringle & Wilson, 1952; Chapman & Smith, 1963); or by means of a small plastic vice upon the armature of a strain gauge dynamometer (Statham G1-4-250) which measured the force applied to the spine. Silver-silver chloride electrodes made contact with the tissue through glass pipettes containing Yeager's saline. Contact with the lumen of the spine under study was made after cutting the end from the spine (Wolbarsht, 1960). The spine electrode pipette, of about 0.5 mm. inside tip diameter, was mounted upon the shaft of a forcing galvanometer (Sanborn 171) and thus also served to transmit forces from the galvanometer to the spine. Potential differences were recorded through a direct-coupled amplifier system with electrometer input (Nagard 2502 or Medistor A-34 modified for battery operation); both were sensitive to about 20 µV. Spine resistances were measured either by shunting the electrodes and preparation with a known resistance (Frank & Fuortes, 1955) or by passing $10^{-9}$ amp. through the preparation.

**Punctate stimulation.** For tactile mapping and related experiments on intact spines, preparations were mounted by pins upon the arm of a Chambers micromanipulator (Text-fig. 1). Punctate stimulation was accomplished usually with a tungsten micro-probe mounted on the armature of a strain gauge dynamometer (Statham G 7B-3-350) operated from a carrier amplifier (Tektronix Type Q), so that the force of probe contact could be monitored throughout the procedure. In addition, the sensory structure could also be stimulated by moving the spine in its socket with a glass rod held in a second micromanipulator (not shown in Text-fig. 1) movements of which could be indicated electrically with a potentiometer connected to a control screw of the micromanipulator. The same glass rod also served to hold the spine against the surface of the leg, in an effort to immobilize the spine, during punctate stimulation with
the probe. Probe force and rod movement were indicated on the lower beam of the oscilloscope (Tektronix 502) by means of an electronic switch (Tektronix Type M).

Afferent discharges picked up from the mounting pins were pre-amplified with a Grass P–8 amplifier using a nominal pass-band from 7 cyc./sec. to 12 kcy./sec., displayed on the upper beam of the oscilloscope, and monitored with an audio amplifier.

Empty glass micropipettes 1–2 μ in tip diameter were first tried as probes for punctate stimulation. While it was possible to stimulate the sensory structure with them, they were too flexible for accurate mapping and too brittle for prolonged use. Satisfactory results were obtained, however, with tungsten probes of 1–1·5 μ minimum radius of curvature at the tip (Plate 2, inset). These were prepared by etching the end of a piece of tungsten wire in molten sodium nitrite heated sufficiently to make the reaction autocatalytic. Under these conditions adequate tips with a specular polish could be formed in a few seconds.

The dynamometer with probe attached was rendered slightly overdamped with a dashpot comprising an empirically determined amount of petroleum jelly partly filling a hole in a piece of plastic through which the shaft of the probe extended (Text-fig. 1). Force calibration was carried out initially with weights suspended from the point of attachment of the probe, then checked routinely with a shunting resistance in the bridge circuit of the carrier amplifier.

For the punctate mapping experiments scales were attached to the X and Y screws of the Chambers micromanipulator to provide reference co-ordinates in the horizontal plane, while the Z screw was used to raise the preparation up against the probe. With each leg preparation it was necessary to calibrate the X and Y scales on the micromanipulator against the ocular micrometer of the dissecting microscope, and to measure the angle between the trajectories of motion produced by the X and Y screws, since in the Chambers manipulator these values depend upon the exact position of the preparation in relation to the stationary parts of the micromanipulator. The position of the preparation in the horizontal plane was subject to a small amount of drift with respect to the X and Y scales, this drift being inherent in the design of the micromanipulator. Drift was only of the order of 3 % when developed in 100 μ movements made in several steps, and in the small-displacement mapping experiments it has been neglected. The slight curvature of motion was barely detectable in the mapping runs, and has also been neglected.

The force of probe contact was adjusted by hand while watching the dynamometer output on the oscilloscope. It was usually possible to make contacts of the order of 100 mg. force with rise-times of 100 msec. or less. Usually the probe and dynamometer were inclined at 30° with respect to the vertical movement of the preparation, in order to facilitate exploration under the crest of the socket of the tactile spine, and no correction for the resolution of the force has been applied in the analysis.

RESULTS

Receptor-potential experiments. In preliminary experiments with seven spine preparations, attempts were made to record receptor potentials from the cut tip of the spine, as described by Wolbarsht (1960). Electrical contact with the lumen of the
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spine was made by progressively clipping distal portions from the spine until the resistance between the tip of the spine and the base of the leg dropped from its initial value of 100 MΩ or more to about 1 MΩ. This usually occurred when between one-third and one-half of the spine had been cut off. Histological comparisons indicate that the lumen usually extends a little more than one-half the length of the spine. When a bending force was applied at this stage, spike activity with apparently normal pulse-frequency modulation characteristics was recorded. Spike amplitudes were of the order of 100–200 μV., as with extracellular recording from the crural nerve in the leg.

No evidence of receptor potential was obtained from any of the preparations, although the recording system could have detected activity down to 10 μV. or less in amplitude. Assuming the resistance of an available longitudinal current path through the lumen of the spine to be 0.5 MΩ, an appropriately distributed generator current of 2 × 10⁻¹¹ amp. or more would have been detected in this experiment. By comparison, Wolbarsht (1960), Morita (1959) and Morita & Yamashita (1961) have obtained receptor potentials of 1–10 mV. or more from insect mechanoreceptors. Wolbarsht's data suggest that his generator currents must have been at least 10⁻¹¹ amp., and the current threshold for a single node of Ranvier in frog nerve may be as high as 10⁻⁹ amp. (Stämpfli, 1954). It is possible, therefore, that the generator current of the present receptor is of comparable magnitude, but does not flow along the lumen of the spine.

It is improbable that receptor potentials were cancelled by interference from the driving signal, since less than 20 μV. of interference was recorded when the preparation was replaced by resistors of comparable magnitudes.

Morphology. Sectioned femoral and tibial tactile spines show the lumen at the base of the spine to be eccentric (Pl. 1 b), with the outer (proximal) cuticular wall appreciably thicker (c. 30 μ) than the inner (c. 10 μ). At the attachment of the spine to the unsclerotized cuticle of its socket, usually in the outermost position, there is a single campaniform sensillum with a cap 10–15 μ across, and a 10 μ diameter canal penetrating the 30 μ thickness of the cuticle, leading to a group of cells in the epidermal layer (Pl. 1 d, e). Among these, a prominent, 8–10 μ nucleus is readily seen under phase contrast, together with elongate 3 × 15 μ nuclei, and smaller ovoid ones. The identity of the corresponding cells has not been established. However, they presumably include a sensory neuron, tormogen and trichogen cells and large epidermal cells (Slifer, 1961; Hsu, 1938). It is noteworthy, however, that methylene blue, usually a successful neuron stain, did stain epidermal cells generally but did not preferentially stain any of the cells at the base of the campaniform sensillum. This point has not been investigated further. Details of the structures in the canal are difficult to resolve in these preparations, as in other insect sensory structures (Slifer, 1961), but a structure presumed to be a scolopale containing the dendritic process of the sensory neuron can clearly be seen in Pl. 1 d. A lip of unsclerotized cuticle of the socket appears to impinge upon the cap of the sensillum (Pl. 1 b, arrow).

In adequately cleared preparations, viewed in cedarwood oil at low magnification, the campaniform sensillum at the base of each tactile spine can be clearly seen as a transparent spot. At higher magnifications in surface view these sensilla often assume a characteristic cardioid shape (Pl. 1 c), with the cusp of the cardioid toward the base
of the spine and pointing toward the tip. It is unclear whether the transverse structure seen bisecting the cap in Pl. 1a and c is a ridge-like thickening of the cap similar to those of the campaniform sensilla of the surfaces of the leg segments (Pringle, 1938) or represents the lip of soft cuticle impinging on the sensillum. In any event, the shape of these sensilla differs in detail from the roughly elliptical ones of the leg surfaces. This is shown in Pl. 1a, in which the sensillum of the first dorsal spine of the tibia may be compared with those of Pringle's group 6 immediately proximal to it (arrows).

In a detailed survey of all spines, 114 in number, of the three left legs of a large (14g.) female, a single campaniform sensillum was found at the base of each spine. In several dozen additional spines from numerous individuals, examined as cleared whole preparations and as fixed sections, no spine has been observed to have more than one campaniform sensillum, nor to have none. In this series, the sizes of the sensilla in surface view ranged from $7 \times 10\mu$ on the spines of the prothoracic femoral fringes to $16 \times 22\mu$ on those of the metathoracic tibia.

**Punctate stimulation.** Punctate mechanical stimulation of the base region of tactile spines was carried out in 21 dorsal femoral and 20 tibial spines in 29 individuals. None of the femoral preparations was subsequently cleared in cedar-wood oil and the location, size and shape of the sensory region, determined by mapping, could not therefore be compared directly with the position and size of the campaniform sensillum in the cleared cuticle. During the probing experiments with live preparations, as with other uncleared material, it was not possible to recognize with certainty any structure resembling the campaniform sensillum. Hence it was impossible to ascertain directly whether a nerve discharge obtained with the probe occurred when the probe was on the sensillum. Moreover, the tip of the probe was often not clearly visible under the dissecting microscope, particularly when close to the surface of the spine, and its position relative to the spine could only be estimated to within about 50 $\mu$. With the glass probes used in the first few probing attempts an added difficulty was that probe shafts could sometimes be seen bending as far as the point at which they became invisible, so that the actual tip position was still less certain. However, it was always possible to demonstrate a highly localized and highly sensitive tactile point on the outer surface of the spine at its base. Impulse frequencies of more than 500 per sec. were occasionally observed immediately upon making contact with the probe, as determined by the minimum interval between action potentials observed at high sweep speeds (faintly visible in Pl. 5c).

Using the tungsten probe it was possible in addition to map out the size and approximate shape of the region, with precision of about 2-5 $\mu$ (Pl. 2). In these mapping experiments the probe was first brought over the preparation visually, using both the coarse micromanipulator holding the probe and the fine manipulator holding the preparation. The preparation was then raised against the probe with about 100 mg. force, for about 1 sec. This was done repeatedly, moving the preparation on each trial until contact produced a discharge in the nerve. Co-ordinates were read from the horizontal screws of the Chambers micromanipulator, and mapping was then carried out by advancing one horizontal screw at a time in small increments so that the probe crossed and recrossed the sensitive region and its surroundings several times in both directions. In each of four of the preparations 50-100 contacts were made in the
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course of 3–5 hr. With each trial probe co-ordinates were recorded, the result was photographed from the oscilloscope (Pl. 3) and, to facilitate the progress of the mapping, points were usually plotted on a grid, indicating the position and result obtained.

Typical responses have been arbitrarily grouped into three classes in Pls. 2 and 3: no discharge (open circles); small discharge, a single impulse or a short burst ceasing before the probe was removed (triangles); and large discharge, frequency of several hundred impulses per sec, lasting at least as long as the applied force, and often followed by an after-discharge several seconds in duration (filled circles).

In the course of the first of the extensive mapping experiments it became evident that the sensitive region underwent drift with respect to the micromanipulator co-ordinates. Two contributing factors appeared to be: (1) movement of the entire spine in its socket, which became obvious when forces as large as 500 mg. were used; and (2) the inherent drift in the micro-manipulator, mentioned previously. To achieve maximum accuracy, therefore, probe forces were not allowed to exceed 100 mg. during a mapping series, and in determining the limits of the sensitive region the total displacement of the micromanipulator was restricted (Pl. 2, rows indicated by arrows).

The best mapping experiments indicate that the most sensitive region extends about 5 μ along the spine axis, and about 10 μ transverse to it. Upon superimposing the sensitivity map upon a photograph of the base of the cleared spine in the same preparation, the sensitive region is seen to agree in size and angular orientation with the campaniform sensillum at the base of the spine. In making this superposition the angular orientation of the map with respect to the photograph was determined from measurements made at the time of the mapping experiment. On the other hand, it was necessary to assign the precise position of the map arbitrarily, because of the uncertainty in the position of the probe. In Pl. 2 this has been done so that the distal and lateral limits of the sensitive zones in the two rows indicated by arrows fall within the edges of the photograph of the sensillum.

The after-discharge which sometimes follows a probe-evoked discharge provides a test which shows that the structure responding to probing is the same one that responds to bending the spine. In eight preparations it was possible to rock the spine during the after-discharge following a probe stimulation. If bending the spine and probing the sensitive spot excited two separate sensory structures, then one would have expected to see nerve impulses evoked by bending interspersed among the impulses of the after-discharge, and their presence would be readily recognized as a separate impulse train, at least by the interval relationships if not by differences in amplitude and wave-form. However, when this was done, bending the spine did not stimulate a second structure but modulated the after-discharge (Pl. 4), showing that the same nerve fibre was stimulated by both manoeuvres.

Action-potential wave-forms evoked by probing and by bending were compared in thirty-eight femoral and tibial spine preparations. Repetitive wave-forms were photographed from the oscilloscope, with the rise of the action potentials triggering the sweep during the mechanically evoked discharges (Pl. 5). A dozen or more superimposed impulses are represented in each photograph.

In all cases, the amplitudes of the action potentials agreed within the limits of variation in a single discharge, and the durations differed by at most a few per cent
for the two methods of stimulation. In twenty-three of the preparations the waveforms obtained by the two methods are virtually identical (Pl. 5a and d), while in the remaining fifteen cases they differed significantly in detail (Pl. 5b and e, c and f). There appeared to be no regularity as to which stimulus evoked the ‘more complex’ wave-form. In all of these preparations the closer recording electrode was usually 3–5 mm. from the base of the spine, so that any electrical activity decrementally conducted from the sensillum might be expected to contribute to the recorded wave-form. However, in the cases where the wave-forms differ, no correlation with the intensity or mode of stimulus has emerged.

Over-stimulation phenomena occurring during punctate stimulation have been observed in many of the receptors (Pl. 6). With the probe on the sensitive region a rather strong stimulus may initiate an intense discharge, followed by a partial or complete inhibition which persists for the duration of the stimulus (Pl. 6a); this may be followed by an after-discharge. If the preparation is re-stimulated during an after-discharge, the impulse frequency may increase with gentle contact, and cease at higher contact forces (Pl. 6c). In the present study no attempt has been made to analyse these results quantitatively. It should be mentioned, however, that a related phenomenon has been observed in some preparations in which the spine was forced outward against its socket with sinusoidally varying forces (unpublished observations); bursts of impulses occurred as the force was released, and ceased as the force was increased. These preparations did not follow the power law transfer function more commonly observed (Chapman & Smith, 1963).

**DISCUSSION**

From the foregoing results I conclude that the campaniform sensillum found at the base of each tactile spine on the legs of *Periplaneta americana* is the sensory structure responsible for the unit discharge that occurs when the spine is touched or moved. The main points of the evidence may be summarized as follows:

1. A single campaniform sensillum was always found at the base of every tactile spine examined, including at least one representative of every tactile spine that occurs on the pro-, meso- and metathoracic legs of this species.

2. A highly sensitive, highly localized region similar in size, shape, orientation and location to the campaniform sensillum can be demonstrated by punctate mechanical stimulation at the base of the spine.

3. Bending the spine during an after-discharge following strong punctate stimulation does not produce a second train of impulses, but modulates the after-discharge. Thus the normal response to bending is produced by the same structure that responds to punctate stimulation.

4. Finally, in more than half of the preparations studied, action potentials evoked by punctate stimulation and by moving the spine, in the same preparations, were equal in amplitude and duration and were indistinguishable in wave-form. In the others, action potentials differed only in certain details of wave-form.

What has actually been proven is that the sensory unit which discharges when the spine is moved is the same as the probe-sensitive one. The mapping data, together with the lack of histological and physiological evidence for any other large sensory structure associated with the spine or its socket, give strong circumstantial evidence
that the probe actually does stimulate the campaniform sensillum. Proof of this point remains incomplete, however, because of the visibility limitation during mapping.

It seems likely that these campaniform sensilla have been previously overlooked simply because tactile spines have been difficult to section, and because the opacity of the base region in vivo and in dried specimens conceals them so effectively. While tetrahydrofuran–Eltringham was the only fixative used in this study, it is very likely responsible for the ease with which the sectioned material could be prepared. It would not be surprising, however, to find that these sensilla have been seen previously in cleared whole-mounted specimens.

The failure to record any generator activity from the cut end of the spine can probably be explained simply in terms of the structural arrangement of the campaniform sensillum. If the generator site of the sensillum is in the dendritic process, as seems likely, then one would expect the greatest density of the extraneuronal generator current to be in the canal in the wall of the spine, not in the lumen proper. If so, it should be possible at least in principle to record receptor potentials between the cap of the sensillum and the base of the spine.

These results suggest further that the distribution of generator current to sites of excitation on the neuron is restricted to the immediate vicinity of the cell group at the base of the canal of the sensillum, and that no appreciable generator current reaches the underlying tissues of the femur. Furthermore, since spikes with typical extracellular amplitudes can be recorded from the cut tip, it is likely that impulse propagation is initiated somewhere within the spine, rather than beyond its base. This is necessarily so if the generator current is confined within the spine.

Differences in the wave-forms of action potentials evoked from a single spine by different methods of stimulation (Pl. 5), and possibly with other conditions of the experiment, have been noted. Some of these appear to be physiologically important, and suggest that the sensory neurons may be capable of initiating impulses at more than one site or in more than one way. Others may be artifacts due to changing the geometry of the preparation or to temporal changes during the course of an experiment.

In all the preparations the distal recording electrode was probably close enough to the base of the spine (often 1.5–2 mm.) to have detected any large, decrementally conducted potential arising within the sensillum itself. The appearance in Pl. 5f but not in Pl. 5e of a second, smaller, downward spike (distal electrode negative) about 0.8 msec. after the initial negative peak may be an example of this. It is conceivable that this represents a somatic spike in the sensory neuron, arising with a low safety factor after the propagated impulse has been initiated at a separate site, in the now familiar manner proposed for spinal motor neurons (Araki & Otani, 1955; Eccles, 1957), crayfish abdominal stretch receptors (Edwards & Ottoson, 1958), and the giant neuron of Aplysia (Tauc, 1962). If so, the occurrence of these delayed spikes is probably related to the intensity of the stimulus or to the frequency of the afferent discharge, and only secondarily to whether the sensillum is stimulated by probing or moving the spine in its socket. In any case, it is very unlikely that the delayed spikes in Pl. 5f originate in a second neurone, because of their short latency and precise synchronization after the first spike.

Wave-form differences as great as those between Pl. 5b and e were occasionally encountered with identical stimuli delivered an hour or more apart. Progressive
dehydration of the preparation is likely to be a cause of these differences. The characteristic shapes of spikes from individual spines, many of which deviate appreciably from the simple diphasic wave-form of the longitudinal action current of a uniformly propagating fibre in a uniform medium, can be attributed to geometric non-uniformities of the tissues of the femur or tibia, and their relationships to the recording electrodes. It is possible that the recording geometry is altered when the spine is moved from its position against the leg for probing the sensillum, and when it is flexed proximally against its socket to evoke discharge by moving it. But since there are many cases in which this factor does not change the action-potential wave-form (e.g. Pl. 5a and d), this possible source of artifact may be unimportant.

The over-stimulation effect (Pl. 6) may represent a conduction failure of the afferent fibre or a failure of the generator mechanism (Eyzaguirre & Kuffler, 1955). Initial impulse frequencies as high as 500 sec.\(^{-1}\) were occasionally observed, while in Pl. 6c and d the discharge ceased at a considerably lower frequency. The distribution of impulse intervals at the onset of failure appears to indicate impulse dropping in some cases, suggesting conduction failure; but this point has not been investigated in detail, and a satisfactory treatment of this awaits generator-current analysis.

The after-discharge that follows strong punctate stimulation may be due to a mechanical hysteresis at the sensory ending, such that deformation of the ending persists transiently even though the probe has been removed, or it may represent an induced transient instability of the sensory ending. In any event, since the after-discharge can be further modulated mechanically, the effectiveness of the structure as a mechanoreceptor is not lost during the after-discharge.

The campaniform sensilla of the tactile spines are evidently stimulated differently from those of the cuticular surfaces elsewhere in the insects; the latter nearly always appear to be arranged to respond to strain in the cuticular wall in which they are embedded (Pringle, 1938). In the tactile spines, they occur in the thickest and presumably least compliant part of the spine wall. In their normal functioning it is highly unlikely that the sclerotized cuticle of the spine is deformed at all, but that the deformation is confined to the soft cuticle of the socket and to the diaphragm which supports the cap of the sensillum. The most likely natural stimulus appears to be movement of the lip of the socket cuticle which impinges on the cap of the sensillum (Pl. 1 b).

Another possible mechanism of mechanical excitation may be by hydraulic transmission of force in either direction along the axis of the canal when the spine is moved normally. However, if the threshold to punctate forces applied to a cap 10 \(\mu\) in diameter is about 10 mg., an equally effective hydraulic pressure from within would be of the order of 10 mg./100 \(\mu\)\(^2\) or 10\(^4\) mm. Hg. This is probably an absurdly high value for the ambient hydrostatic pressure in a body cavity, since the pressures which provide motive force in much smaller spider legs are less than one atmosphere (Parry & Brown, 1959). However, the force measurements made on the abnormally positioned spines under experimental conditions may give a distorted impression of the compliance of the cap of the sensillum under natural conditions. Moreover, forces occurring within the tissues of the cockroach leg are almost certainly not purely hydrostatic, so the possibility of a piston-like displacement of the cap cannot be entirely ruled out on the basis of this crude force analysis.
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SUMMARY

1. The failure to record receptor potentials from cut ends of the large tactile spines of the legs of the cockroach *Periplaneta americana* has prompted a histological and electrophysiological search for the sensory unit of these tactile spines.

2. Histological sections, made with a cuticle-softening fixative containing tetrahydrofuran, and cleared whole-cuticle preparations reveal a single campaniform sensillum in the thick cuticular wall of the spine at its junction with the soft cuticle of its socket.

3. Punctate stimulation in the same region reveals a highly mechanosensitive region similar in size, shape, orientation and location to the campaniform sensillum.

4. Movement of the spine during after-discharges which sometimes follow punctate stimulation does not produce a second impulse train, but modulates the after-discharge.

5. It is concluded that the campaniform sensillum is the sensory structure of the tactile spines of the cockroach, and is responsible for the normal discharge that occurs on contact with the spine.

6. Strong stimulation causes a failure of the afferent discharge, resembling the 'overstretch' phenomena seen with other mechanoreceptors.

7. Spike wave-form analysis suggests that impulse initiation may occur at more than one site on the sensory neuron or in more than one manner, and includes the possibility of a delayed, non-propagated discharge of the soma of the sensory neuron.

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EXPLANATION OF PLATES

**PLATE 1**

(a) Cleared preparation of metathoracic tibia, showing the campaniform sensillum in the base of the first dorsal spine (arrow at right) and the sensilla of Pringle's group 6 just proximal to it (arrow at left). Most of the latter are not in focus.

(b) 15 μ oblique section through the base of the dorsal tactile spine of the metathoracic femur prepared with tetrahydrofuran-Eltringham fixative, under phase contrast. The campaniform sensillum is in the thickened proximal (upper) wall of the spine at its junction with the unsclerotized cuticle of its socket. The femur extends proximally to the left of the picture, the tibia distally to the bottom. Magnification as (a).

(c) Surface view of the campaniform sensillum of the corresponding spine in a cleared specimen, photographed in cedarwood oil through the overhanging crest of the socket, with small condenser aperture. Spine pointing toward upper right.

(d) Same section as (b), focused on the cap and the presumed dendritic process of the sensory neuron in the canal. Magnification as (c).

(e) Same, focused on a prominent cell nucleus in the epidermal layer opposite the canal of the sensillum. Magnification as (c).

**PLATE 2**

Sensitivity map of the sensory structure at the base of the dorsal spine of a metathoracic femur (array of plotted points), superimposed upon a photomicrograph of the campaniform sensillum of the same preparation after clearing (Pl. 1c). Filled circles, triangles, and open circles indicated sensitivity in decreasing order, corresponding to records in Pl. 3. Further details in text. Inset shows the tip of the tungsten probe used in this experiment, at the same magnification.

**PLATE 3**

Discharges obtained during the mapping experiment of Pl. 2, classified according to magnitude of response to less than 100 mg. probe force, corresponding to symbols used in Pl. 2. Each record; upper trace, afferent discharges; lower trace, force applied by the probe. Calibrations all as in uppermost record.

**PLATE 4**

Test for the identity of probe-evoked and movement-evoked discharges, 4th dorsal spine of tibia. Each record, top to bottom: afferent impulses, amplitude calibration at left; movement of tactile spine, uncalibrated; 10 msec. time intervals, faintly visible below base-line; force of contact of tungsten probe, calibration at left. (a) Two movements of spine, each of which evokes a train of impulses. (b) The two film records are continuous; calibrations as in (a). 300 mg. probe contact for about ½ sec. on the point of maximum sensitivity (upper record) evokes a train of impulses followed by an after-discharge. Moving the spine three times before cessation of the after-discharge (lower record) does not evoke a second unit discharge, but modulates the after-discharge, indicating that the same unit is stimulated by both manoeuvres.
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Spike wave-forms during repetitive discharges obtained by manipulating spines (a, b, c) and by probe contact (d, e, f) respectively, in three different preparations. Each frame: upper trace, superimposed spike wave-forms, amplitude calibrations same in (a) and (d), (b, c, e, f) as in (b); lower trace, probe force; time and force calibrations all as in (a). (a, d) and (c, f) dorsal femoral spines; (b, e) 5th dorsal tibial spine. Spike amplitude and duration are essentially the same in each preparation with the two methods of stimulation, as are the wave-forms in preparation (a, d). In (b, e) and (c, f), probe-evoked wave-forms differ in detail from those obtained by manipulation.

Overstimulation phenomena, dorsal femoral spine. Traces as in Pl. 3. (a), Response ceases abruptly during strongly effective stimulation, yet a prolonged after-discharge ensues when the probe is removed. (b, c, d) Continuous records of a prolonged after-discharge in the same preparation, evoked by probe contact in (b). In (c) very gentle contact (about 20 mg.) during the after-discharge sharply increases the discharge frequency; next, stronger force abolishes the response, and the after-discharge resumes as the stimulus is removed. In (d) probe contact again interrupts the after-discharge.