

Analysis of the Neural Responses of
a Cockroach Trochanteral Tactile Hair
to Mechanical Stimulation

by

Hugh Spencer

A Thesis

Submitted to The Faculty of Graduate Studies
In Partial Fulfilment of The Requirements For The Degree
of Master of Science

Department of Physiology

Winnipeg, Manitoba

September 1971



ABSTRACT

Both adult and juvenile cockroaches of the species Periplaneta americana of both sexes possess prominent hair sensillae on the ventral surface of the proximal segments of all thoracic legs which, when the animal is at rest project perpendicularly downwards from the plane of the animal's body. In this position they would appear to have a possible role as detectors of surface vibration, or irregularities as the animal is moving about. The sensillae could also function as contact chemoreceptors.

This study was undertaken to elucidate the response characteristics of these receptors, with a view to both establishing their function and determining the nature of the receptor mechanisms involved.

Receptor responses resulting from sinusoidal and ramp mechanical displacement stimuli were analysed using a range of techniques, including the averaging of instantaneous rates of receptor firing and cumulative impulse counts using an averaging computer. An exponential regression analysis of the cumulative impulse count of the responses of the receptor to ramp stimulus displacement was carried out using an IBM 3600 computer.

Attempts were also made to record the responses of the hair sensillae in freely walking animals in order to determine their physiological role.

The receptor response to mechanical deflection is a phasic high frequency (700 pps) burst of firing, and the

receptor did not exhibit chemosensory responses. Impulse firing synchrony to the stimulus is maintained at frequencies in excess of 300 cps, and the receptor has a relatively slow time course of habituation.

The number of impulses generated in response to a given velocity mechanical stimulus is a power function of the stimulus displacement, and all receptors obey this function closely ($P > 0.005$).

Unfortunately, the physiological role of the receptor hairs could not be established.

It appears that the method of analysis employed in the study may permit analysis of the different components involved in the receptor responses.

ACKNOWLEDGEMENTS

I wish to thank Dr. K. Hughes and Dr. N. Clinch, of the Department of Physiology, for support and loan of apparatus; Dr. T. Dandy and Dr. C.K. Govind, of the Department of Zoology, for the loan of an oscilloscope and camera; and Dr. C. Pinsky, of the Department of Pharmacology, for use of equipment.

I wish also to thank Mr. R. Kapitany, of the Department of Oral Biology, for supplying the tape recorder used in this study.

I especially wish to thank my wife, Barbara, for her invaluable assistance in the statistical analysis of the data, and for proof reading this dissertation.

TABLE OF CONTENTS

	Page
INTRODUCTION	2
Basic structure of Hair Sensillae	3
Mechano Receptive Hair Sensillae	4
Chemoreceptors	6
Response Characteristics	7
AIM OF THE STUDY	
MATERIALS AND METHODS	10
Experimental Animals	10
Experimental Preparation	10
Stimulus Systems	12
Photographic	15
Tape	17
Analysis System	20
Instantaneous Rate Analysis	20
Averaged Cumulative Count Analysis	24
Receptor Potential Recording	25
Freely Walking Preparation	27
Chemical Stimulation	29
ANATOMY	
Distribution of the Sensillae	32
Anatomy of the Sensillae	32
RESULTS	34
Responses to Sinusoidal Mechanical Stim- ulation	34
Responses to Ramp Stimulation	41

TABLE OF CONTENTS (CONT'D.)	Page
Qualitative	41
Quantitative	43
Stimulus Velocity	43
Stimulus Displacement	44
Determination Stimulus-Response relationship	46
Receptor Potentials	58
Tonic Displacement Receptors	59
Thermal Responses	60
Freely Walking Preparation	60
DISCUSSION	67
BIBLIOGRAPHY	71
APPENDIX	
1. Regression curves and data points for each set of receptors analyses	75
2. Instantaneous rate meter	76
3. Cumulative pulse counter	78
4. Ramp generator	80

LIST OF PLATES AND FIGURES

Page

Plate

- | | |
|--|----|
| 1. Postural attitudes of resting cockroaches | 1 |
| 2. Stereoscopic photograph of cockroach leg | 13 |
| 3. Sample of data frame | 18 |

Figure

- | | |
|---|----|
| 1. Schematic of initial experimental system | 16 |
| 2. Schematic of system employed to tape records | 19 |
| 3. Analysis system using the CAT 400 | 20 |
| 4. Comparison between the stimulus, receptor firing pattern and the outputs of the analysis methods | 23 |
| 5. Receptor potential recording system | |
| Receptor potential from a trochanteral sensillum | 26 |
| 6. Ventral view of thoracic leg showing position of tactile hairs | 30 |
| 7. Responses of the trochanteral hair sensillum to bursts of sinusoidal mechanical stimuli | 31 |
| 8. Responses of the trochanteral hair sensillum to bursts of sinusoidal mechanical stimuli | 37 |
| 9. After discharges following sinusoidal stimulation | 38 |
| 10. Responses to frequency swept sinusoidal mechanical stimulus | 39 |
| 11. Time course of habituation of a trochanteral hair | 40 |
| 12. Averaged instantaneous firing rates for a trochanteral hair | 42 |

LIST OF PLATES AND FIGURES (CONT'D.)	Page
Figure	
13. Average initial firing rate of a trochanteral hair sensilla versus ramp stimulus velocity	45
14. Averaged cumulative counts for responses of the trochanteral hair sensillae to ramp stimuli of varying amplitude and velocity	47
15. Diagram illustrating the effect of the normalizing procedure	48
16. Log-log plot of normalized responses of preparation B10 to ramp stimulus displacement	50
17. Log-log plot of normalized responses of preparation C2 to ramp stimulus displacement	51
18. Log-log plot of normalized responses of four different animals to a wider displacement range	52
19. Scatter diagram of the normalized responses of 12 sensillae	55
20. Film records of receptor potentials in response to mechanical stimuli	61
21. Tonic responses of secondary receptor to prolonged extreme mechanical displacement of the hair	62
22. Responses of the preparation to radiant heat	63
23. Continuous film recording from unrestrained animal	65
24. Continuous film recording from unrestrained animal	66

Plate 1. Two postural attitudes commonly assumed by resting cockroaches. (*Periplaneta americana*)

Top. Low position - in this position the trochanteral and perhaps the femoral hair sensillae are in contact with the substrate, in which position they could act as substrate vibration receptors - this position is often observed in resting healthy animals, and is also a characteristic posture of animals in poor condition.

Bottom. Raised position - this is the more commonly seen resting posture, and the sensillae are lifted well clear of the substrate. When the animal is feeding or moving about they also assume essentially the same posture.



INTRODUCTION

Arthropods differ from most other organisms in having a rigid chitinous exoskeleton, which serves both to protect the animal's internal environment, and as an attachment for the musculature required for movement.

Normally this exocuticle is non-living and rigid, as indeed it must be, and considerable pressures relative to the animal's size are required to deform it.

Since an insect must have information about the nature of the environment in which it exists, there must exist mechanisms whereby external stimuli can be detected and transduced and the information made available to the CNS.

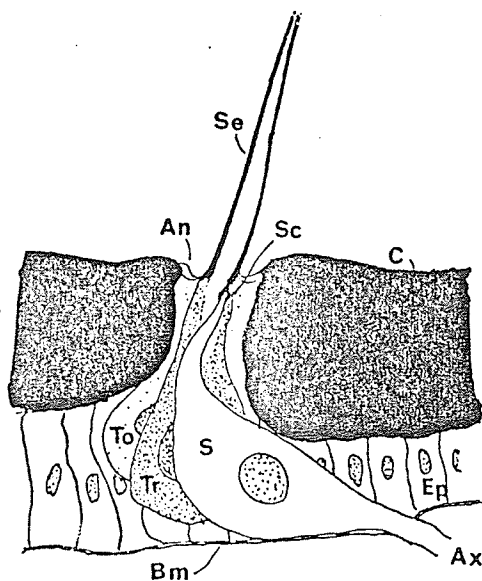
To provide the necessary sensitivity in the presence of the exocuticle, the insects have evolved a vast array of projections and modifications of the exocuticle, which function as transducing windows in the animal's 'shell'. Transducing windows because the projections and modifications usually serve to translate one variable, such as displacement into another, distortion of a surface, and so permit the sensory cells to encode this as a train of impulses.

Such windows are most numerous on the perimeter of the animal, those parts which make the initial contact with the environment - the antennae, head, legs and tip of the abdomen. Large numbers also surround the articulating surfaces of every joint, and these are generally held to be proprioceptors (Dethier 1963).

Basic Structure of Hair Sensillae

Central to all peripheral receptors is a modified cuticular area. This modification may be in the form of a hair or peg (sensilla trichoida), a plate (sensilla placodea), a thin domed region of the cuticle (campaniform sensilla), or an open tipped hair (chemo sensory). It is generally assumed (Snodgrass, 1935) that the archetypal sensilla pattern is that of the hair sensilla, being derived from setae, and that other sensillae are in turn derived from it.

Essentially the hair sensillae consist of a long hollow bristle of varying rigidity inserted into an annulus of membranous exocuticle surrounded by a ring of thick exocuticle. The membranous annular ring functions both as a fulcrum and to provide a restoring force to the sensilla after the disturbance which caused the deflection has passed. In many hair sensillae, the leverage ratio inherent in the structure is such that the neural transducer proper behaves as an isometric force transducer, while the tip of the hair behaves as an isotonic level. The displacement division ratio can be in excess of 200:1 in very long hairs.



- An Annular ring of thin cuticle
- Ax Sensory cell axon
- Bm Basement membrane
- C Cuticle
- Ep Epidermal cells
- S Sensory cell
- Sc Scopopoid sheath
- Se Setae
- To Tormogen or socket cell
- Tr Trichogen or hair forming cell

Diagrammatic anatomy of Sensilla trichoidea (after Snodgrass 1935)

In the simplest sensilla trichoida, only one sensory neurone is found, and such hair receptors are generally simple mechano receptors. However more than one sensory neurone may be present.

In general the sensory neurone, which is considered to be a primary sensory neurone (Dethier 63), is bipolar, the point of insertion of the spical dendrite region being at varying distances up the hollow hair shaft. Associated with the dendrite of the sensory neurone is a tubular and possibly chitinous sheath, the scolops, which appears to be a consistent feature of all sensillae derived from the archetypal trichosensilla (Dethier 63). Associated with the sensory neurone are at least two other cells, a trichogen, or hair forming cell, and a tormogen or socket forming cell.

As previously mentioned the responses spectrum of the receptor can be determined partly by the structure of the 'hair', and partly by the characteristics of the receptor region of the neurone. Thus a number of sensilla trichoida which appear to have essentially the same structure, may have totally different stimulus-response characteristics.

Mechanoreceptive Hair Sensillae

Cahmi (1969) has described a sensory hair of the simple sensilla trichoida type on the head of locust, Schistocerca gregaria. This hair which possessed a single sensory neurone, was found to be a wind velocity receptor; the neurone firing tonically for a sustained deflection of the hair. The receptor

firing frequency was a linear function of the wind velocity. In addition to being a tonic receptor, this receptor showed a high degree of directional sensitivity by virtue of the curvature of the seta and an asymmetric compliance of the socket.

In contrast a tarsal tactile hair described by Runion and Usherwood (1968) from the same animal has a similar structure, but the response of the receptor is a phasic burst of impulses following sustained mechanical displacement.

A major problem encountered during the present study has been the dearth of studies on insect mechanoreceptors. Most workers have concentrated on insect chemoreceptors or on the mechano and proprioceptors of other arthropod classes, chiefly Crustacea. However the basic mechanisms would appear to be similar (Laverack 1963, 1966; de Forrest Mellon 1963; Wyse 1965; Sanjeeva-Reddy 1971).

In many ways the insect sensilla trichoida mechanoreceptor is analogous to the tylotrich and monotrach sensillae in mammals (Iggo 1968). The hollow setae are analogous to the hair itself, and the point of insertion of the mammalian hair into the epidermis provides both a fulcrum and a restoring force as does the annulus of the tricho sensilla. The basket of free nerve endings which surround the base of the mammalian hair and the dendrite of the tricho sensilla both perform the transduction of the movement into neural impulses. There also appears to be considerable parallels in the diversity of the stimulus response spectrum, and in the presence of multiple

neural endings encoding different parameters of the stimulus.

Both Lowenstein (1958) and Eyzaguirre and Kuffler (1955) have demonstrated the nature of the generator potential in the Pacinian corpuscle and Crustacean stretch receptors respectively. No comparable work appears to have been carried out on mechanoreceptors in insects. However, Wolbarsht (1960) recorded what he referred to as a 'receptor' potentials from the cut end of mechano sensory hairs in a number of insects, in response to mechanical deformation. The magnitude of the receptor voltage recorded ranged from 1 to 10 mV, and was either transient or sustained in response to sustained deformation of the hair, depending on whether the receptor was of a fast or slow adapting type. However the polarity of the receptor potential is negative, in contrast to that observed by intracellular recording. Wolbarsht has concluded that this extracellular recorded potential represents the potential difference between the dendritic and spike generating regions of the receptor neurones. He also concluded that the spike was generated proximally to the receptor site and did not invade the receptor region and also demonstrated that spike frequency was a linear function of receptor potential amplitude.

Chemo Receptors

A number of simple chemo-receptors are anatomically very similar to the tricho-sensilla mechano receptor. In many cases tactile tricho-sensillae also possess one or more supernumerary neurones, the dendrites of which pass through the lumen of the seta to terminate at an opening at the tip.

These have been shown to be chemosensory receptors which are capable of responding to chemical substances ranging from aromatic compounds, amino acids, pherones to water (Dethier 1963).

Response Characteristics

Werner and Mountcastle (1965) demonstrated that the mammalian mechanoreceptive endings described originally by Iggo (Iggo's Corpuscles), exhibited a stimulus-response relationship which was a linear relationship between the log of the response and the log of the stimulus magnitude. In other words the receptor response was a power function of the stimulus magnitude.

Unfortunately very little quantitative work has been carried out on insect mechanoreceptors, and most of that which has been carried out has been of a somewhat cursory nature.

Usherwood (1968) working with a chordotonal proprioceptor from the tibia-femur joint of the locust Schistocerca, found that this receptor encodes the tibia-femur angle on either side of a centre point as an increasing firing frequency with a high degree of linearity, but with directional ambiguity. The response was comprised of two components, a phasic component with changing angle, and a slowly adapting component proportional to the final angle.

Unfortunately the structure of the insect chordotonal organs, while considered to be derived from the original tricho sensillum (Snodgrass 1935), is considerably different

from that of the hair receptors, although the basic transduction mechanism is, in all probability, the same.

To date the most thoroughly analysed insect mechanoreceptor is the campaniform organ that underlies the large tibial and femoral spines on the legs of cockroaches. These spines, often incorrectly classified as trichosensillae, are relatively massive (Fig. 1), and hinged at the base in such a way that attempts to move them back forces a projection at the base of the spines against a chitinous stop. As a result the spines act as ratchets, allowing the animal to increase its traction when pushing past obstacles.

A campaniform sensilla situated near the stop is distorted when the spine base bears upon it, and functions as an isometric force transducer (Chapman 1965).

Several workers (Pringle and Wilson 1952; Chapman 1963; and Crowe 1967) have analysed this receptor in great detail. The primary aim of their studies has been to develop a transfer function of the stimulus-response relationship in order to permit the prediction of the receptor's responses to time varying signals of the sort encountered in nature. In fact this preparation has become a proving ground for techniques of linear analysis aimed at deriving mechano receptor transfer functions (Holden 1971). Most of its value in this respect lies in its relatively complex responses to a stimulus, as well as its simplicity and the ease of recording responses.

Crowe has shown that the peak firing frequency of this

receptor is a linear function of tension over the range 0.2 to 2.5 g wt., when determined by using ramp or sinusoidal driving functions. However the slope of the relationship, is to some extent also a function of the velocity of the driving waveform, and the linearity does not hold at low stimulus values.

As yet no worker has attempted to determine the aspects of the receptor responses that are physiologically significant to the animal, a task which could prove to be formidable, since we have no intuitive knowledge of the sensory environment the insect dwells in. When Werner and Mountcastle (1965) attempted to do this for a mammalian touch receptor (Iggo's Corpuscle), they had the assistance of a considerable body of psychophysical theory on sensory discrimination from which to select models that could be tested from the neurophysiological data.

As a result of this lack of theory, many of the characteristics of the receptor responses that have been analysed by various workers may in fact be of little or no consequence to the animal, but however, may provide extremely valuable information about the actual transduction mechanisms involved.