THE RESPONSES OF MECHANORECEPTORS OF THE TIBIAL AND FEMORAL SEGMENTS OF THE COCKROACH LEG

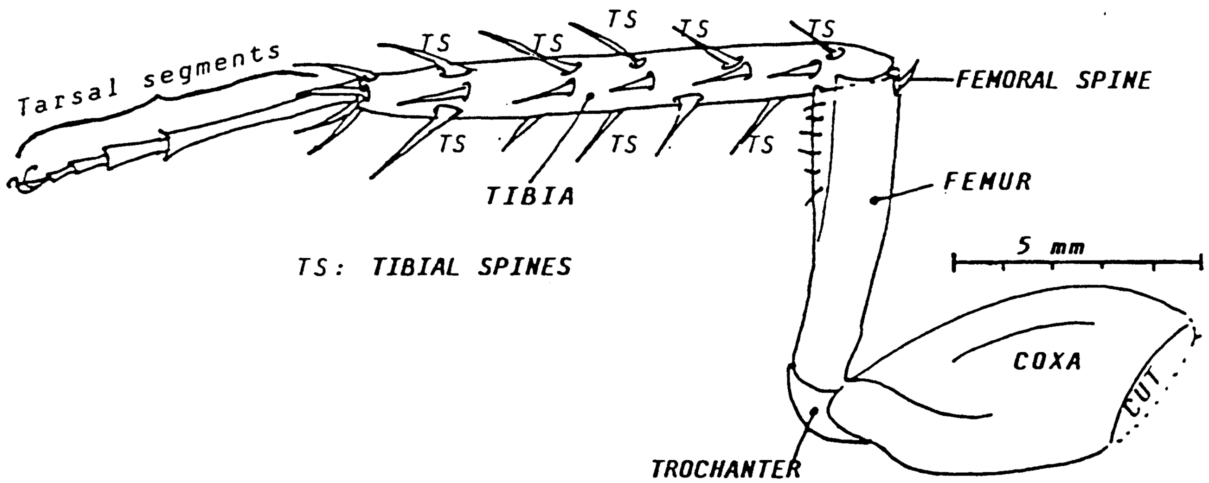
Aim:

- to record neural activity
- to show that mechanical input leads to neural output
- to study adaptation of the neural response
- to devise your own experiment

Introduction

The legs of most insects bear bristle-like sense organs, which are able to transduce mechanical into electrical energy in the form of trains of action potentials. These are primarily concerned with the assessment of position and movement of the animal, and rely partly upon contact with the ground and partly on chance collisions with the objects in the environment for their stimulation. They are not the only form in which mechanoreceptors are found; some longer, very thin hairlike sense organs (called trichobothria) are found in spiders where they detect air currents and airborne vibrations. The cockroach has similarly sensitive receptors on its two abdominal cerci.

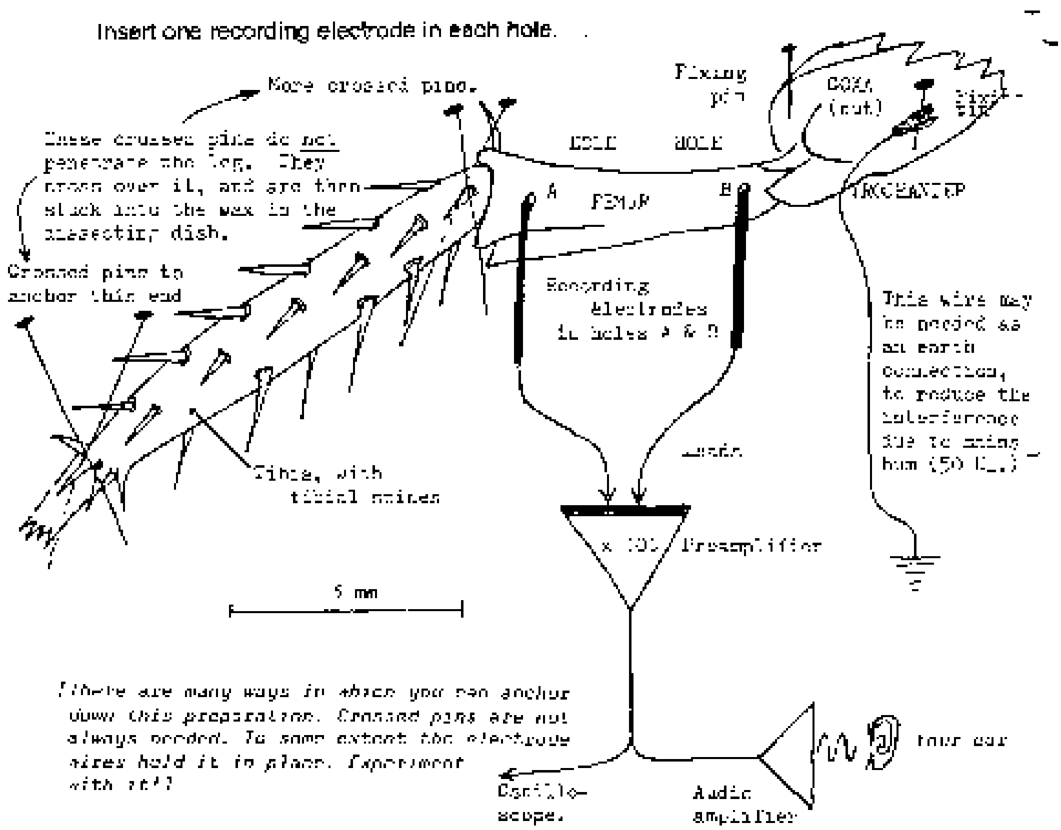
We shall investigate the responses of some well-marked, large spiny mechanoreceptor sensilla. (The term 'sensilla' means 'sense organs' and is often applied to types occurring in arthropods). The spines are located as shown in **Figure 1.** We will record the electrical responses (OUTPUT) of the sensory cells located at the bases of these spines by placing recording electrodes on the leg's main sensory nerve trunk where it passes through the femur. The procedure is straightforward. Indeed, it is among the simplest preparation known , but it still allows you to record the responses of **single** sensory cells. Stimuli (INPUT) are made by bending the spines through an angle in their sockets.



The Preparation

Anaesthetise a cockroach with carbon dioxide. When it has succumbed, cut off one of its the hind pair of legs by cutting straight through the coxa, (next to the thorax). Do **not** cut through the femoral segment. Set the femur in a piece of blutack with one of its flat surfaces downwards. Extend the tibial segment of the limb and fix that down also, with more blutack, though you need to keep the spines free to move.

Arrange a stereomicroscope to view the preparation. Using the microscope to observe your movements, make two small holes in the cuticle of the femur, one towards each end of the segment. You will probably find that the silver electrodes are sharp enough to make the holes, but only puncture the cuticle on one side because you will damage the femoral nerve if you push right through the femur.



The preamplifier is connected to the recording electrodes and to the oscilloscope. In addition, it feeds signals to a PC and audio-amplifier so that you can 'hear' the responses of the sensory cells. Check that the oscilloscope is on and that the beam is running across the screen in free-running mode.

You can use the computer to store electrical traces of sensory responses. The cockroach program (icon on the right) is a standard windows program with menus to sample, set a spike threshold and to save the traces. You can reopen files with the **.wav** extension in the program later Please save the traces into the s:\junk directory so I can send them to you later! The File | Save Picture command also saves a version with the **.emf** extension and this is a picture which can be imported into your word-processed account.

Experimental

Quantitative experimentation with sensory cells is very difficult under 'class' conditions, but you could try to find out whether there is any mechanical directionality in the spine's response and whether it is phasic or tonic in response to angle change as follows.

To move a spine, the simplest way is to insert a mounted needle into the blutack near one of them. It could be the terminal femoral or one of the bigger tibial spines. Next, bend the spine one way **[NB Make sure you also gently hold the baseplate to keep the hum down]** or another and both **look** at the oscilloscope screen and **listen** to the audio-amplifier to find the response of the sensory cell attached to that spine. You may like to mount the end of the pin in a spare micromanipulator.

On the oscilloscope screen, or from the computer-recorded traces you may well see the responses of more than one sensory cell. However, any individual cell, or 'unit' (as it is sometimes called) may be identified by the constancy of size of its own individual recorded action potentials, which differs in size from those of other cells, unless you are unlucky!

The computer-calculated, **frequency-time** plot is simply a convenient way of showing pulsed responses in graphical form. Frequency (*1/time*) is calculated for each interpulse interval and then these values are plotted against elapsed time.

In practice we find that the impulse frequency of most *tonic* receptors, or the tonic part of a mixed *phasic-tonic* response is proportional to the logarithm of the stimulus intensity **S** which is above the threshold stimulus, $\mathbf{S_t}$

Thus: impulse frequency $f = k \log(S\text{-}St) + f_u$ where f_u is the unstimulated response frequency. Usually, this is zero for this preparation.

Please ask for help if you are confused. I think we can sort out most of it by help with the experiment directly, or other advice. This was one of the first sensory systems to be investigated in detail electrophysiologically, by Pringle and Wilson in 1952.

This experiment is open ended; it is intended that you plan you own experiment. Here are some questions you might aim to answer.

- **Is the spine more easily deformed in some directions than in others?**
- **Is the response to movement phasic (i.e. does it generate impulses at a higher frequency if you bend it faster)?**
- **Does the receptor show a tonic response to a fixed (held) angle of deformation in any part of its range of movement?**
- **Are there sectors of angular spine movement where alt the response is phasic, and the impulse generation stops when you stop moving the spine, even though it is not in its resting position?**
- **Do all spines behave the same?**

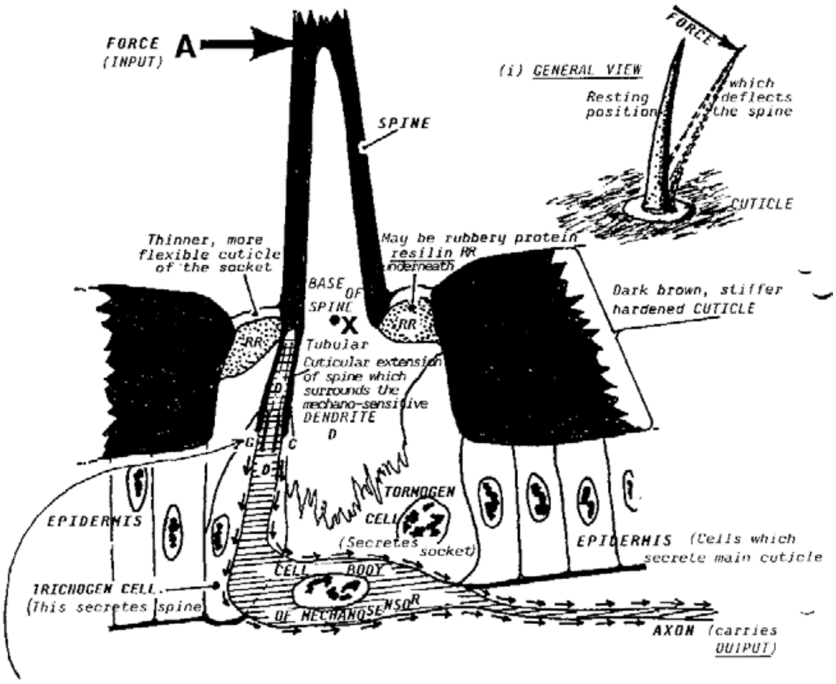
Commentary

The sensory cells associated with these spines detect mechanical stimuli, as opposed to those, which are chemical, electrical, thermal, or photic in nature. The **stimulus** is a force applied to the spine, which causes it to move in an angle and deform its socket in the cuticle. Detection of the stimuli are shown by **responses** caused in which trains (sequences) of action potentials per unit of time are generated by the sensory cell. Responses seen as action potential (AP) sequences are best thought of in terms of the number of APs generated per unit time - I.e. their frequency.

The size of a stimulus can be quantified as the **angle** through which the spine is bent from its resting position.

How does it work?

Well, approximately by means of the mechanism involving cells and cuticular components which is shown in **Figure B2.**



Action potentials generated here at membrane around G and then pass along the cell body membrane and axon in the direction of the small arrows, towards the femoral nerve (where you record them) and further on into the thoracic CNS ganglia. (ii)

Figure 3 Vertical section through base of spine, showing associated cells (Magnification of cells is exaggerated.)

The spines work as levers; when a spine is deflected about point X (near its base), the thin cuticle of the socket is compressed on the same side as that from which bending force is applied, but is stretched on the opposite side. Rubbery protein (resilin), working like a spring, may be present in the socket so that the spine can return to its resting position when the stimulus is removed.

Now, the **dendrite** of the sensory cell is embedded within a thin-walled cuticular tube (the scolopale) extending **asymmetrically** from one side of the spine base. The dendrite is the **transducer of mechanical into electrical energy** and the **initial generator of action potentials in a response from** region G in the Figure. The dendrite is only depolarised, in these sensilla, if it is **compressed.** So, if you apply stimulus force A in the direction shown, you will compress and depolarise it, via the compression of the fine cuticular tube. Force applied in the opposite direction will not cause excitation.

What do the sensory cells tell the CNS?

1. Receptors may be directionally sensitive.

- What follows may seem awkward but it applies, in one way or another, to all biological sensors found in multicellular animals. It is therefore of very general relevance to sensory physiology.

2. Receptors, like man-made sensors, may indicate the strength of a stimulus or only respond to changing stimuli.In other words, they may repsond to

1. Stimulus ***level*** (size, intensity, concentration etc.), **As does a** spring-balance or a thermometer
2. Stimulus *rate of change*, **as does** a speedometer.

BUT Many sensory cells add a further complication. During a period of response, **part** of it may signal rate of change of stimulus and the **rest** will indicate the stimulus level which has been reached. This is a difference from many man-made sensors.

Three kinds of receptors?

Tonic cells are LEVEL sensors, which generate responses which are **electrical analogues** strictly of the SIZE of the stimuli and which have the **same time-course. These are called TONIC RESPONSES.**

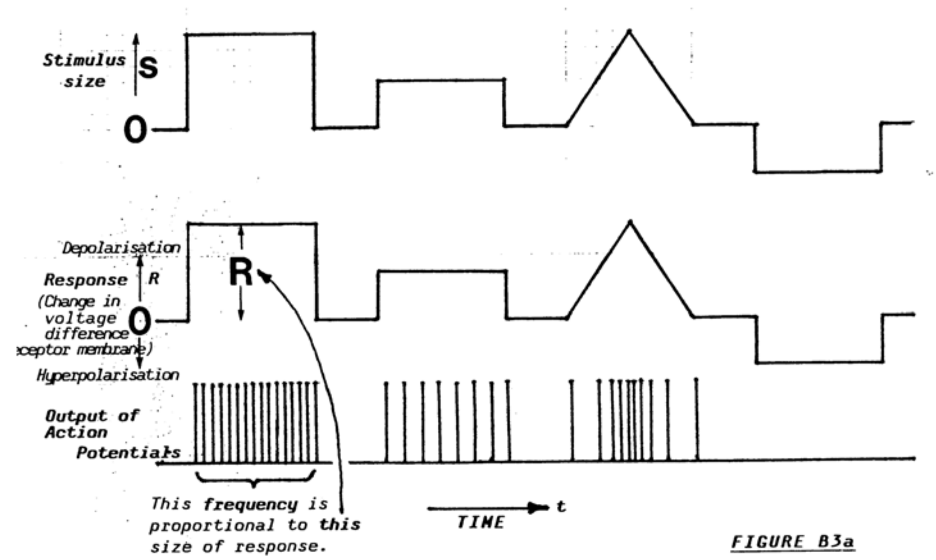


FIGURE B3a
TONIC RECEPTORS (measure 'LEVELS')

There will be **a fixed value of response R whilst the stimulus S is kept at the same level.** Otherwise R follows the same time-course as S (as a thermometer or fuel-gauge)

So, R = f(S) where f() means 'a function of.

Phasic sensors are RATE sensors generate responses which are electrical analogues of the **RATE OF CHANGE** of the stimulus size, S. Here, the response R will be **zero** when the level of S does not change, **(quite unlike** level sensors). These are called **PHASIC RESPONSES**

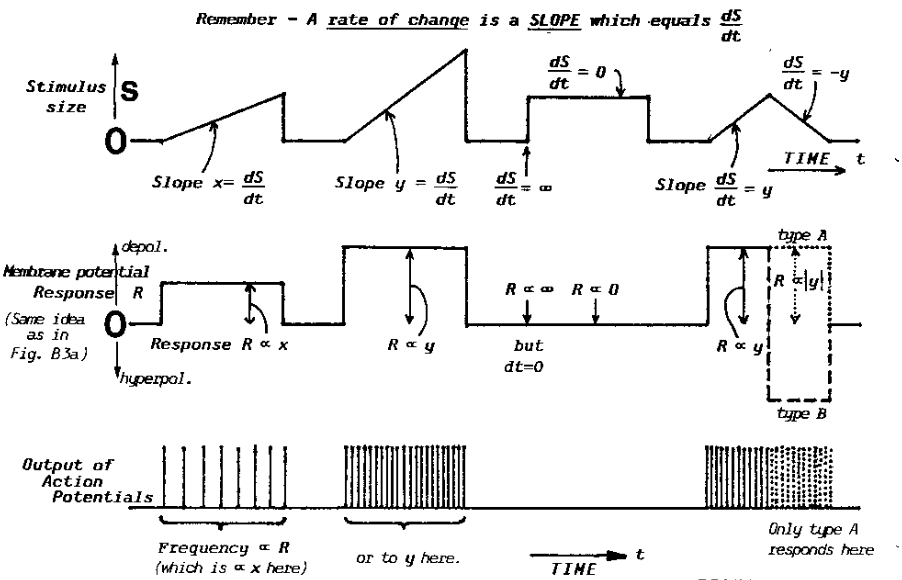


FIGURE B3b

PHASIC RECEPTORS (measure 'RATES')

There will only be a **(fixed) value of response R if** the **stimulus S is changing at a (constant) rate** (as in a speedometer).

So, R **=f(as/at)** [or, maybe, R = f (d^2s/at^2) etc., as would be the case for an accelerometer, or the vertebrates' Pacinian corpuscle, a pressure receptor].

Also, if the receptor is affected by whether change is **increasing** or **decreasing,** it would respond like Type B in **Figure B 3b** and shut off when the change reversed. Otherwise, if **not** affected, it would go on responding to change, even though it had reversed from increase to decrease in S, as in Type A

Phasic-Tonic I mentioned earlier on [in Section 2 (iii)] that a sensory cell may first respond PHASICALLY (rate sensor) and later TONICALLY (level sensor) in time sequence.

Not surprisingly, these responses are called **PHASIC-TONIC** !

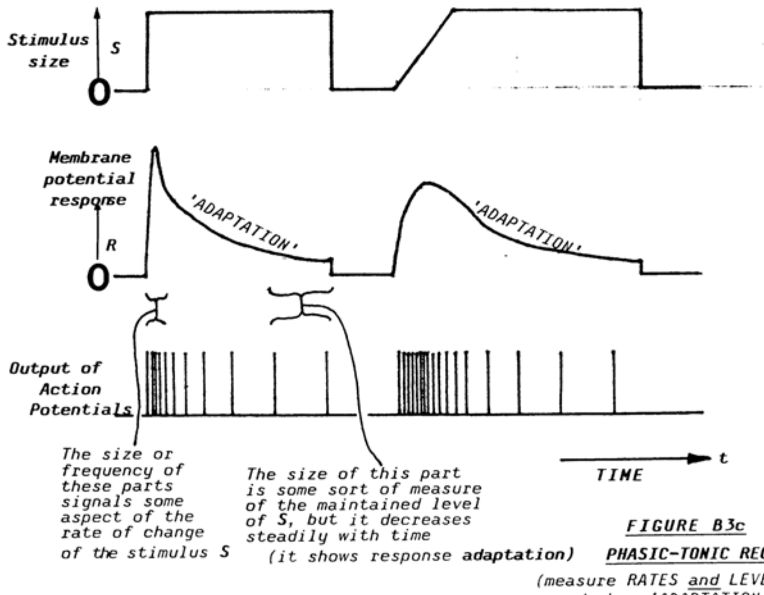


FIGURE B3c

PHASIC-TONIC RECEPTORS

(measure RATES and LEVELS, and show 'ADAPTATION' of response in between.)

The word **'adaptation"** is here in yet another of its ambiguous alternative usages.

The **adapting response** (as it is called) to a stimulus which contains both change and constancy during its existence is possibly a sign of economy of sensory axon commitment. Insects have rather limited numbers of neurons, and the cuticle is pierced by quite small numbers of sensilla, which avoids some mechanical weakening Although unproven, it seems reasonable to suggest that one neuronal channel is better used to conduct three sorts of information (about *rate, level and direction*) than just one. However, 'adaptation' (in this sense) may alternatively be a sign that a sensory neuron simply can't keep up the initially high rate of action potential generation because of energetic cost or perhaps limits on the rate at which the sodium-potassium ion exchange pump can work in a sensory cell.

We can test these cockroach spine sensilla to see whether they are sensors of level or of rate, or both, and also whether their responses are directional

Mechanoreceptors in the cockroach leg tend to be phasic-tonic, but some are almost entirely phasic in response. The stimulus here could be a sudden application of force to the spine, as you would get by bending it suddenly into a new position and then releasing it equally suddenly to rest. This is the sort of stimulus the insect would get from walking into a small obstacle.

References

- French, A.S. (1988) Ann. Rev. Entomol. 33: 39-58 (a good review worth reading)
- French, A.S. (1984) J.Comp. Physiol. 155: 803-812 (He has written numerous other papers on this sense organ.)
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- Kuster, et al. (1983) Proc.Roy.Soc.B,219: 397-412