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RECEPTORS IN THE BILL OF THE PLATYPUS

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SUMMARY

1. Afferent responses were recorded from filaments of the trigeminal nerve in each of two platypuses (*Ornithorhynchus anatinus*) anaesthetized with α-chloralose. All receptive fields were located along the lateral border of the upper bill. Discrete receptive fields could be identified as belonging to two distinct classes of sensory receptor.

2. The most prominent response was an irregular resting discharge which could be increased or decreased by weak electric pulses. These receptors were insensitive to moderately strong mechanical stimulation, and it was concluded that they were electroreceptors.

3. Each electroreceptor had a single spot of maximum sensitivity on the bill surface. When the stimulating electrode over this spot was the cathode it excited the receptor for the duration of the stimulating pulse, using stimulus strengths as low as 20 mV. When it was the anode, it inhibited the discharge. Cathodal excitation was followed by rebound inhibition and anodal inhibition by rebound excitation.

4. Receptors responded to cathodal steps with an initial high-frequency burst of impulses, followed by a lower maintained rate of discharge. Rapidly changing pulses were similarly effective in exciting receptors, adding support to the claim that platypuses are able to detect moving prey by the electrical activity associated with muscle contraction.

5. The centres of the receptive fields of two electroreceptors were marked by the insertion of fine entomological pins. Histological examination established the presence of a large mucus-secreting gland at the marked spot. The epidermal duct of the gland contained an elaborate myelinated innervation, with morphologically distinct axon terminals that we identify as the electroreceptors.

6. As well as electroreceptors, the skin of the bill contained three kinds of mechanoreceptors: slow-adapting receptors, rapidly adapting, vibration-sensitive receptors and receptors with an intermediate adaptation rate. The slowly adapting receptors were characterized by their low threshold to mechanical stimuli, irregular discharge and significant dynamic sensitivity. Vibration receptors showed maintained responses to sinusoidal vibration of the skin up to 600 Hz.

7. These experiments confirm an earlier report that the platypus bill is an electrodetector organ. The presence of electroreceptors of a unique structure and

supplied by the trigeminal nerve indicates that electroreception has evolved independently in monotremes. This in turn emphasizes that monotremes are a highly evolved group which split off from the main mammalian stem a long time ago.

INTRODUCTION

The two representatives of the monotremes, the platypus and echidna can be distinguished from other mammals by a number of unique characters, several of which point directly to the reptilian origins of the group. While a considerable amount of information has been compiled about the behaviour and reproductive biology of these animals, very little is known about sensory mechanisms. As a first step towards obtaining more information on this subject, a systematic analysis was carried out on receptors in the echidna snout (Iggo, McIntyre & Proske, 1985). Now we have extended the work to include a study of receptors in the bill of the platypus.

The platypus bill would seem a structure of particular interest to sensory physiologists because of its rich innervation and the profusion of specialized receptor organs. Indeed as long ago as 1802 Home described the olfactory and optic nerves of platypus as small while the fifth nerve was 'uncommonly large'. This account was later supplemented by descriptions of numerous specialized nerve terminals (see Bohringer, 1981, for references). More recently interest in the platypus bill was renewed when reports were published of its structure and innervation using modern histological methods (Bohringer, 1981; Andres & von Düring, 1984). These revealed elaborate end-organs of several different kinds, richly innervated, which were thought to be principally mechanosensitive, concerned perhaps with the detection of static and dynamic stimuli associated perhaps with the flow of water.

However, the platypus which has remained for so many years one of the greatest zoological curiosities has yielded yet one more surprise. It had been remarked on many occasions that the bill of the platypus had to be of particular importance as a receptor organ since it was known that platypuses shut eyes, ears and nose during each dive, and are often encountered inhabiting turbid water (Barrett, 1941). Now behavioural observations and electrophysiological measurements have been reported which indicate that the platypus is able to detect electric fields with its bill (Scheich, Langner, Tidemman, Coles & Guppy, 1986). The electrodetection system is sensitive enough to signal the presence of weak DC fields as well as rapidly changing potentials associated with muscular activity in moving prey. It therefore seems possible that the platypus receives quite detailed information about its underwater surroundings from the electric fields detected by receptors in the bill: in the diving platypus its conventional 'special' senses are relegated to a relatively minor role.

The receptor structures which are most likely to have an electroreceptive function are the 'gland duct' receptors (Andres & von Düring, 1984). The 'push rods' are presumably mechanoreceptors. Push rods are only found in monotremes but they bear some resemblance to the Eimer organ of moles (Quilliam, 1966; Andres & von Düring, 1973). The platypus bill is thus endowed with two kinds of specialized receptor organs whose function has so far only been inferred from comparative studies of other vertebrates. The electrophysiological measurements of Scheich *et al.*

(1986) were restricted to gross electrical stimulation across the bill and recordings of the activity evoked from the somatosensory region of cerebral cortex. Recordings of afferent activity coming from the bill were not attempted.

We describe here the first recordings of the activity of afferent fibres supplying skin of the bill of the upper jaw of platypus. It was possible to identify receptors which showed a selective sensitivity to either electrical or mechanical stimuli. This study therefore provides direct confirmatory evidence for the existence of electroreception in monotremes. The discharge properties of some afferents supplying mechanoreceptors are also described although the precise function of the push rods remains the subject of speculation.

Preliminary reports of this work have already been published (Gregory, Iggo, McIntyre & Proske, 1986*a*, *b*, 1987).

METHODS

The platypus (*Ornithorhynchus anatinus*) and the echidna are the two living members of the group Monotremata. The platypus lives in small streams in temperate parts of eastern and southern Australia. Because of its secretive habits and because it is widely known that at the beginning of this century platypuses were slaughtered in large numbers for their skins, platypuses are now thought to be rather rare. In fact, the informed naturalist knows that their numbers have recovered considerably and nowadays they can be readily located in most suitable streams.

The animals used in the two successful experiments were caught by net, at night, by the investigators themselves and were brought directly into the laboratory the following morning. It is essential to do this since platypuses appear to be easily stressed and adult animals rarely survive for long in captivity. Since it was known that the experiments would last 18–20 h it was essential to obtain animals in as near perfect a condition as possible.

The two animals, a 0·7 kg female and a 1·5 kg male were given an intraperitoneal injection of α-chloralose (60 mg per kg, BDH). This was supplemented with an intramuscular injection of a mixture of seven parts (by mass) Ketamine (ketalar, Parke-Davis) to three parts Xylazine (Rompun, Bayer), 7·5 mg kg^{-1}, to accelerate induction of anaesthesia. Once the animal was sufficiently deeply anaesthetized a tracheal cannula was inserted. At this stage breathing became progressively slower and more irregular so that it was necessary to put the animal on an open-circuit respiration pump (Palmer) with a ventilation rate of 25–30 per min. The rate and depth of ventilation were adjusted until the expired CO_2, measured with a Datex Normocap CO_2 monitor, reached a steady level of 5%. The animal was placed on an electric heating blanket with feed-back control from a thermal probe inserted into the cloaca. Rectal temperature measured as 32 °C at the beginning of the experiment could be maintained within one or two degrees for the duration of the experiment.

Once a stable level of anaesthesia had been reached this was maintained for most of the experiment. Only in one of the two animals was it found necessary to give a supplementary dose of anaesthetic. However, to ensure that an adequate depth of anaesthesia was always being maintained, at regular intervals the flexor withdrawal reflex threshold was tested by pinching the skin of the forelimb or of the bill. Because it was known that these animals were particularly prone to stress, 4 mg of dexamethasone was injected intramuscularly to maintain blood steroid levels. Throughout the experiment the electrocardiogram was monitored by means of electrodes attached to the skin of the limbs.

The infraorbital branch of the trigeminal nerve was exposed on the left side after collapsing the eye and removing part of the temporal bone. A small glass plate was placed under the nerve and fine strands dissected from it. The infraorbital nerve is very large in the platypus and a number of large blood vessels run within it. It was therefore not possible to transect it entirely and strands had to be removed from near the exposed surface. This meant that activity was being sampled from only one region of the nerve. The dissected nerve strand was placed over bipolar platinum electrodes and the recorded signal was amplified and filtered to be displayed on either a storage oscilloscope

(Tektronix 5000 series) or a digital oscilloscope (Nicolet 2010). On occasions it was found convenient to display not the impulses themselves but their instantaneous frequency. Before conversion to a frequency display impulses were fed through a time–amplitude window discriminator (Bak Electronics). The frequency record was displayed on the digital oscilloscope and then stored on 'floppy' diskettes. Directly recorded trains of action potentials were stored in the same way. Some of the frequency records from the Nicolet were, for further analysis, electronically transferred to a Tektronix 4051 desk-top computer. This was programmed to find the pulses, check the height and timing of each pulse for consistency and produce statistics and histograms of frequency and interpulse interval from the height of the pulses. Statistics of frequencies and intervals were produced separately since the mean frequency is not in general equal to the reciprocal of the mean interpulse interval.

Mechanical stimuli were applied to the skin surface by means of a Ling type 101 electromagnetic vibrator driven by a signal generator which provided ramp-and-hold waveforms of varying rise-times and amplitudes, as well as sine waves over a frequency range of 1–1000 Hz. However, since the movement of the vibrator was not regulated by feed-back, the amplitude of displacement of the loaded probe tip attached to the vibrator fell off steeply at frequencies above 300 Hz. Actual displacement of the probe tip during an applied waveform was measured under a microscope, using stroboscopic illumination to measure high-frequency oscillations. The probe consisted of a 5 cm long nylon shaft which tapered at one end to a 0·5 mm diameter tip. The other end was screwed into the coil of the vibrator.

Electrical stimulation of the bill surface was effected by means of a pair of platinum or silver wire electrodes connected to an isolated stimulator (Grass SIU5). On some occasions, stimulus current was measured by monitoring the potential difference across a series resistor. Both step-function and ramp-shaped stimulating pulses were used. In order to keep skin resistance at a low value the bill was regularly irrigated with tap water and at times a water-soaked pad of cotton was applied to its upper surface.

Some attempt was made to estimate conduction velocity in individual afferent fibres. The site of maximum sensitivity of the mechanoreceptor or electroreceptor was located and a sufficiently strong square-wave pulse applied with the cathode over the sensitive area, to elicit an electrically evoked action potential in the nerve. The conduction distance was taken to be the distance in a straight line between the stimulating cathode and the first recording electrode.

Whenever activity in a strand of nerve could be reduced so that a single functioning axon could be identified, the region of maximum sensitivity was carefully plotted using weak cathodal pulses or mechanical stimuli. The site of maximum sensitivity was marked on a map of the bill. In one animal, for two electroreceptors, fine entomological pins were placed in the skin of the bill, on either side of the point of maximum sensitivity. At the end of the experiment the animal was then killed by an overdose of anaesthetic and perfused with 10% formol saline. The skin of the bill was subsequently processed for histological analysis at both the light and electronmicroscope level, particular attention being paid to the sites marked by the pins. The marker pins were removed after the pieces of bill skin containing them had been cut out from the fixed skin. The skin blocks were embedded in Araldite and semi-thin and thin sections were cut and mounted on electron-microscope grids. They were then examined with a Phillips 400B electron microscope.

RESULTS

Fine strands of nerve contained a mass of spontaneous activity and it was therefore necessary to dissect down to even smaller filaments to try to restrict activity to one or two afferents. It soon became apparent that receptors generating the resting discharge were not mechanically sensitive, that is they were unaffected by moderately strong probing or vibrating of the skin surface. They were, however, responsive to weak electric pulses applied between a pair of stimulating electrodes in contact with the bill surface. When the surface of the bill was systematically tested using different locations for the stimulating electrodes, it was found that for each receptor responsive to electrical stimulation a single spot of maximum sensitivity

could be localized. The area covered was less than 1 mm^2 (Fig. 1), and only slight displacement from this spot resulted in a steep increase in threshold.

Although the sample of afferent units obtained from the two animals was rather small, sixteen electrosensitive units and eight mechanoreceptive units, at no time was there any difficulty in distinguishing between the two receptor types. With one electrode over the sensitive spot, electroreceptors could be excited by cathodal pulses

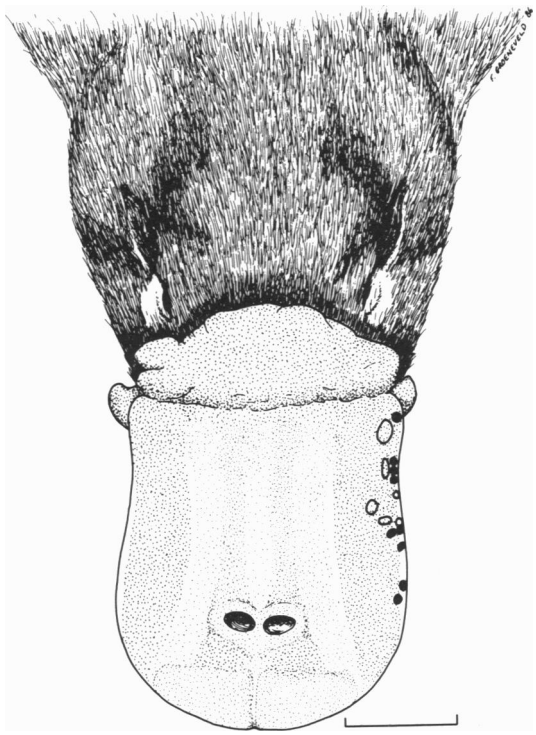


Fig. 1. A drawing of the head of an adult male platypus indicating the locations of electrosensitive spots (filled rings) and receptive areas for mechanoreceptors (open rings). Calibration bar 20 mm. The actual size of individual electrosensitive spots was less than 1 mm^2 but has been increased here for clarity.

of 20 mV or less. None of the mechanoreceptors responded to electrical stimulation across the bill unless stimuli were used which were two to three orders of magnitude stronger than that required for adequate excitation of electroreceptors. Furthermore the sample of mechanoreceptors in this study contained no units which had a resting discharge in the absence of mechanical stimulation.

A drawing of the bill with locations of receptive fields is shown in Fig. 1. The actual sensitive spots for electroreceptors were smaller than indicated, the size of the spots having been increased for clarity. However, all receptive fields for electroreceptors were punctate, any individual afferent unit being excited from a single spot when tested with a fine wire electrode, while for some mechanoreceptors a larger field could be delineated. All receptive fields lay on the lateral border of the bill, due, probably,

to the known high density of receptors in this region (Andres & von Düring, 1984) and because sampling of afferents was restricted to the lateral edge of the nerve trunk. An attempt to assess the relative frequency of occurrence of each receptor type suggested that the majority of afferents in at least this portion of the nerve were electroreceptors. However, the prominence given to afferent activity of electroreceptors by the presence of a resting discharge may have led to a biased impression.

Electroreceptors

While the majority of electroreceptors showed an irregular resting discharge, silent units were occasionally encountered (Fig. 5). Some receptors responded with regularly repeated bursts (Fig. 2) and it occurred to us that this might be due to weak currents generated by the recording equipment. However, there was no simple correlation between the burst frequency and the 50 cycle mains frequency. Nor were

Fig. 2. 'Spontaneous' bursting discharge of an electroreceptor. The burst frequency was not directly related to the 50 cycle mains frequency, respiration rate or heart rate.

the bursts linked with the respiratory rhythm or heart rate. It is known that electroreceptors of some fish respond to the animal's own respiratory movements (Bullock, 1982).

The resting activity of electroreceptors could be decreased by irrigating the bill surface with warm tap water. In this respect the platypus receptors resemble the ampullary receptors of elasmobranchs which behave like mammalian cold receptors (Murray, 1974). When physiological saline was used instead of tap water to irrigate the bill surface it led to a transient increase in discharge, perhaps as a result of the increase in external sodium concentration. It is known that ampullary receptors increase their discharge when the sodium chloride concentration in the surrounding sea water is raised (Loewenstein & Ishiko, 1962).

The resting discharge of electroreceptors lay in the range 20–50 impulses s^{-1}. The mean rate for each of six units measured from successive intervals over a 10 s period was 27, 28, 32, 43, 43 and 46 impulses s^{-1}. The coefficient of variation lay in the range 0·4–0·6. For each response a histogram was drawn showing the interval distribution (Fig. 3). Intervals were not uniformly distributed about the mean (cf. Münz, Class & Fritsch, 1984), and the distribution was skewed towards the long-interval end.

It was possible to modulate the background discharge of an electroreceptor afferent according to the polarity of the electrode. Provided the stimulating voltage was large enough, any site of electrode placement on the surface of the bill could be

used to elicit a response. Nor did it matter what were the relative positions of the anode and cathode except that it determined the polarity of the stimulus which excited the receptor. When the cathode lay closer to the point of maximum sensitivity, the receptor was always excited (Fig. 4). An anodal pulse decreased firing and if made strong enough could silence the discharge throughout the pulse (Fig. 4).

As mentioned before, if the surface of the bill was carefully explored with weak voltage pulses using one fixed and one roving electrode, a point of maximum

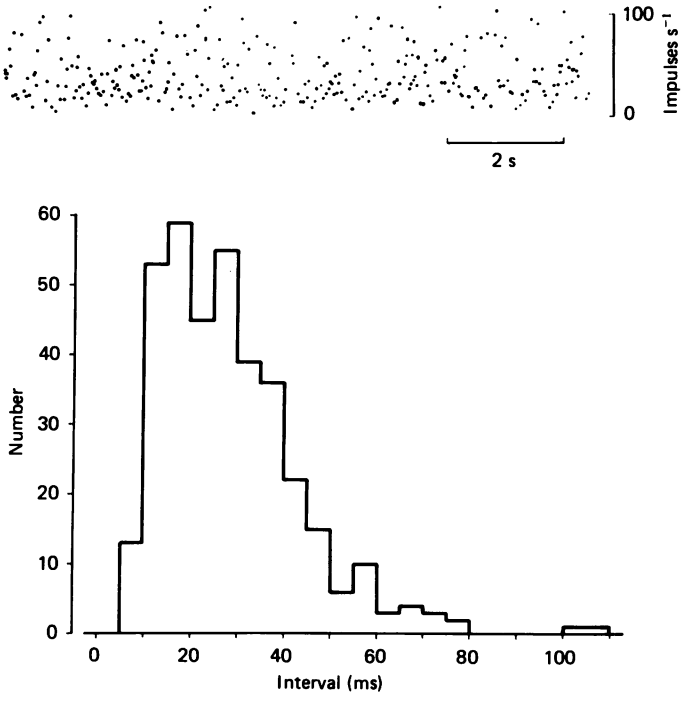


Fig. 3. A typical example of the resting discharge of an electroreceptor (upper panel), displayed as instantaneous frequency to emphasize the irregularity of the discharge. In the histogram below is shown the distribution of 367 successive intervals recorded over a 10 s period. Intervals were grouped into 5 ms bins. The mean interval was 29 ms and the coefficient of variation 0·53. This unit had a mean firing rate of 46 impulses s^{-1}.

sensitivity could be located where a suprathreshold cathodal pulse elicited a high-frequency discharge from the receptor (Fig. 5). The unit illustrated in Fig. 5 was a rare example of a receptor with no resting discharge. When stimulus polarity was reversed and the anode lay over the sensitive spot, if the receptor had a resting discharge then it was silenced by the pulse but this was followed by a large rebound excitation. Excitation was followed by a period of rebound inhibition. Powerful effects such as that illustrated in Fig. 5 were not seen if the roving electrode was displaced only slightly from the point of maximum sensitivity.

A systematic study of the stimulus–response relationship was carried out for cathodal and anodal pulses of various amplitudes on three electroreceptors. All measurements were made with one electrode over the sensitive spot. It was found

that it required pulses of 15–20 mV to obtain a detectable modulation of the resting discharge. Measurements of skin resistance gave values of about 1 MΩ. Threshold current was estimated to be about 0·02 μA.

The relationship between the size of a 2 s voltage pulse and the mean afferent firing rate is shown in Fig. 6. This particular receptor had a resting discharge rate of 27 impulses s^{-1}. An anodal pulse of 20 mV was enough to silence the discharge. In response to cathodal pulses of 20 mV or more, afferent firing during the pulse increased above the basal level. With stronger pulses up to about 40 mV firing rate increased steeply up to a mean rate of 90 impulses s^{-1}. Further increases in stimulus

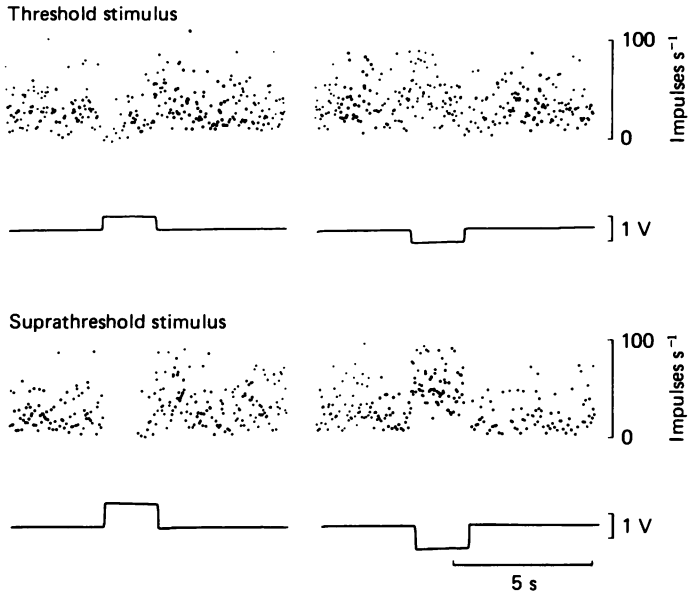


Fig. 4. Instantaneous frequency display of the resting discharge of an electroreceptor, modulated by anodal pulses (left-hand panel) and by cathodal pulses (right-hand panel). Afferent discharge increases during cathodal and decreases during anodal stimulation. In each record the upper trace represents the afferent discharge and the monitored stimulating pulse is shown below. Upper pair of records, threshold stimulus; lower pair, suprathreshold stimulus. The two stimulating electrodes were placed 5 cm apart, antero-posteriorly on the bill surface and were equidistant from the point of maximum sensitivity. A downward deflection indicates the electrode nearest to the tip of the bill was the cathode.

strength produced even higher firing rates but the rate of increase of discharge was more gradual. The response appeared to reach a maximum value of 270 impulses s^{-1} using a 330 mV cathodal pulse. Additional increases in stimulus strength did not raise the rate of discharge further, nor was there any sign of a significantly reduced response with large cathodal pulses (cf. Fig. 5, Teeter, Szamier & Bennett, 1980).

In the response illustrated in the lower panel of Fig. 5, there was an initial high-frequency burst of impulses during onset of the square-wave cathodal pulse and firing was at a sufficiently high rate to reduce briefly the amplitude of the recorded

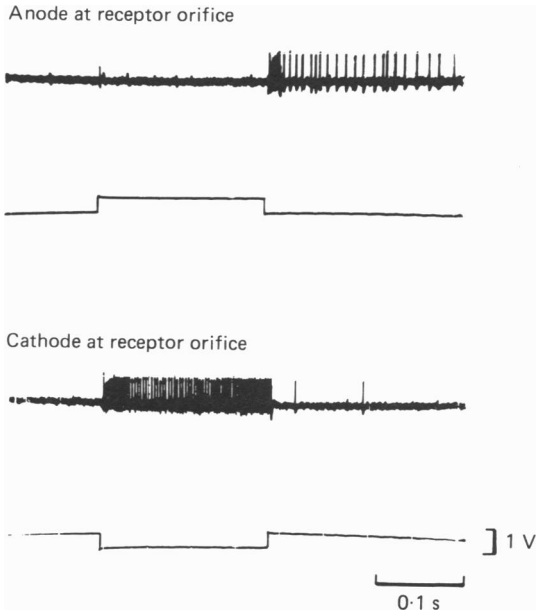


Fig. 5. Responses of an electroreceptor to anodal pulses (upper pair of traces) and to cathodal pulses (lower pair). Upper trace in each pair, impulse discharge; lower trace monitored voltage pulse. Note that this was a rare example of an electroreceptor with no resting discharge. The electrodes were 1 cm apart with one electrode lying directly over the point of maximum sensitivity. Because of the lack of a resting discharge the only detectable effect of anodal stimulation was rebound excitation (upper panel).

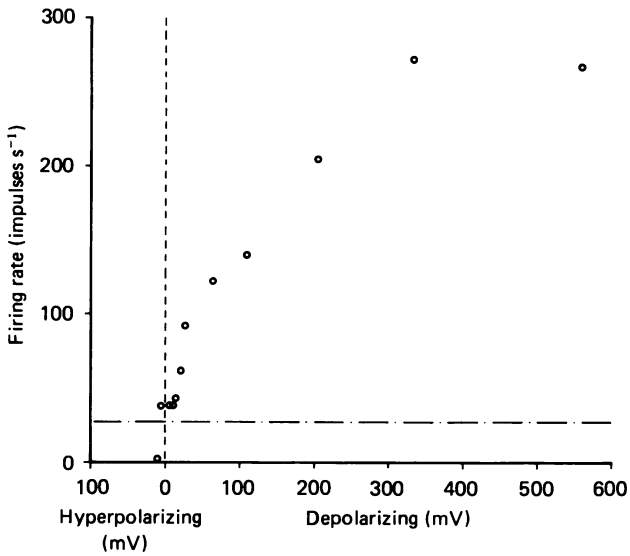


Fig. 6. Mean afferent firing rate of an electroreceptor in response to 2 s cathodal or anodal pulses of different amplitudes. The horizontal dashed line indicates the basal firing rate which for this unit was 27 impulses s^{-1}. The vertical dashed line locates the point of no stimulation.

potentials. Subsequently firing slowed to a more-or-less steady maintained level. The presence of some adaptation during the voltage step suggested that the electroreceptors had a significant rate sensitivity. It was therefore decided to test the effect on the discharge of voltage pulses with rapid rise times.

The observations of Scheich *et al.* (1986) had suggested that platypuses are able to detect rapidly changing pulses such as that associated with the tail flick of a freshwater shrimp. We therefore tried stimulating with a brief voltage pulse shaped to correspond approximately to that recorded by Scheich *et al.* for a shrimp tail flick (Fig. 7). The pulse consisted of two triangular pulses at a frequency of 150 Hz. It was

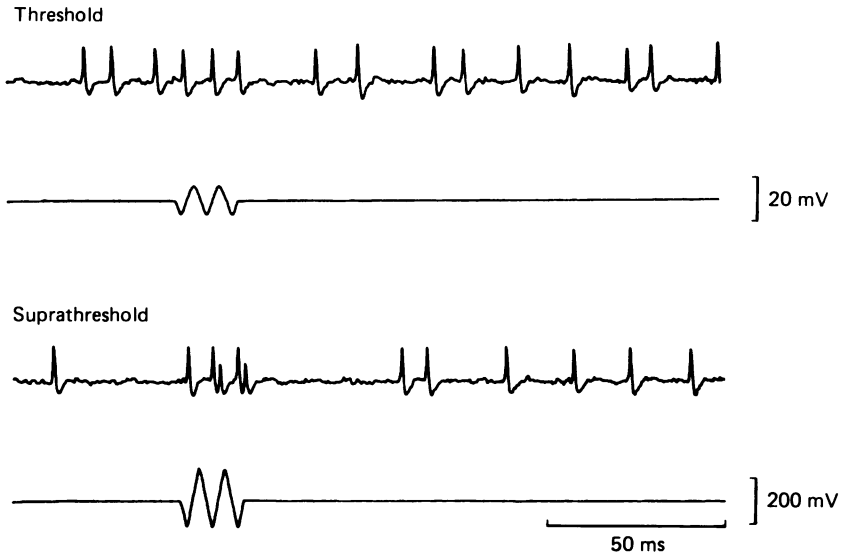


Fig. 7. The response of an electroreceptor to a voltage pulse with a shape arranged to resemble the field potential recorded near a shrimp flicking its tail (see Scheich *et al.* 1986). Upper panel, threshold response (14 mV); lower panel, suprathreshold response (220 mV). In each panel action potentials are shown on the upper trace and the monitored voltage pulse below. In the lower panel the smaller action potentials following two of the larger potentials are thought not to be a second unit coming in, but the first unit firing at a sufficiently high rate (500 impules s^{-1}) for the impulse amplitude to become reduced.

found that such a waveform was able to cause a detectable modulation of afferent firing, using a peak-to-peak amplitude of only 14 mV, which was as low as any of the values for threshold measured using square-wave stimulation. Increasing the size of the pulse produced a larger response (lower panel, Fig. 7), the unit now responding with two impulses per voltage pulse at a rate of 500 impulses s^{-1}.

Histology of electroreceptors

The skin of the bill of a platypus that had been fixed by perfusion at the end of an electrophysiological experiment was processed for electron microscopical examination. The tracks of the marker pins were clearly visible in the semi-thin sections and as illustrated in Plate 1*A* were found to bracket a large mucus sensory

gland duct (using Andres and von Düring's terminology). The gland was cut longitudinally in successive sections of the tissue and was examined in ultra-thin sections. The two receptors marked during electrophysiological recording were at different places on the margin of the upper bill and each of them corresponded to a mucus sensory gland. At the epidermal border there is an accumulation of myelinated axon profiles (MA) and just below in the dermis there is a small group of six myelinated axons (N). Some of the axonal profiles at the base of the epidermis are more superficial and a more highly magnified view of an axon in that position is seen in Plate 1 *B*. There are several features of particular interest. First, the axon is still myelinated, with about thirty-five myelin lamellae. Second, there is a remarkable infolding of the axolemma accompanied by the plasma membrane of the Schwann cells innermost lamellae. Third, there is a small gap in the myelin through which penetrates a slender filament of the axon that is free of any investing Schwann cell. The axonal protrusion is in contact with cells of the wall of the mucus sensory gland's duct. This tongue of axon represents the terminal of the sensory nerve fibre and is likely to be involved in the transduction process. There is insufficient information to decide whether the afferent fibre ends in a single terminal or branches at the base of the gland to form several endings. The electrophysiological evidence however points to a single gland containing the terminals of only a single afferent fibre.

Mechanoreceptors

It was possible to study mechanoreceptors only when all spontaneous activity coming from electroreceptors had been eliminated from the recording filament. Receptive fields of mechanoreceptors were all small (cf. Bohringer & Rowe, 1977) and in the sample studied lay close to the lateral border of the bill (Fig. 1). Of the eight mechanoreceptors encountered, one was lost before it could be studied in detail, while the others were classified according to their rate of adaptation during a maintained stimulus. They included two slowly adapting receptors, three which were rapidly adapting and two which had intermediate properties. Two of the rapidly adapting receptors were called vibration receptors because of their high vibration sensitivity.

The responses of a slowly adapting receptor are shown in Fig. 8. The receptive field was on the edge of the bill and very discrete, about 2 mm diameter. Small-amplitude indentation of the skin triggered a high-frequency burst of impulses which declined to a slower maintained rate. A 20 μm indentation was sufficient to trigger a response (Fig. 8). A twice-threshold stimulus elicited firing rates up to 500 impulses s^{-1}. Ramp and hold indentations of the skin with different rise-times showed that this receptor had a pronounced dynamic sensitivity. The maintained discharge during the hold phase was rather irregular. This particular unit responded to vibration with a 1:1 response up to 300 Hz. A cutaneous mechanoreceptor, slowly adapting, with velocity sensitivity and an irregular discharge is known in other mammals as the cutaneous slowly adapting type I (SAI) or Merkel receptor (Iggo & Muir, 1969).

An example of a mechanoreceptor with intermediate adaptation rate is shown in Fig. 9. This receptor had a relatively low mechanical threshold, (25 μm), and responded at threshold with three impulses. A maximal stimulus produced an irregular spluttering discharge which was maintained for only 1–2 s. This receptor

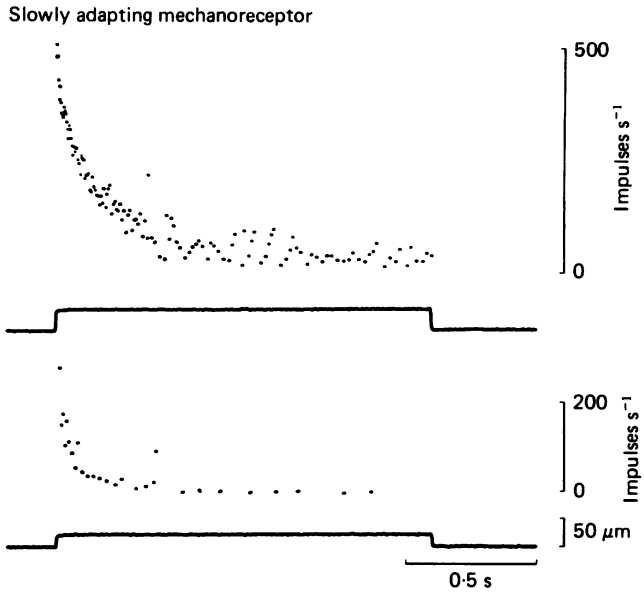


Fig. 8. Slowly adapting mechanoreceptor responding to step indentation of the skin in the receptive field. In each panel the upper trace is an instantaneous frequency display; the lower trace is the monitored movement. Bottom panel, threshold stimulation (20 μm skin indentation); upper panel, suprathresold stimulus (40 μm).

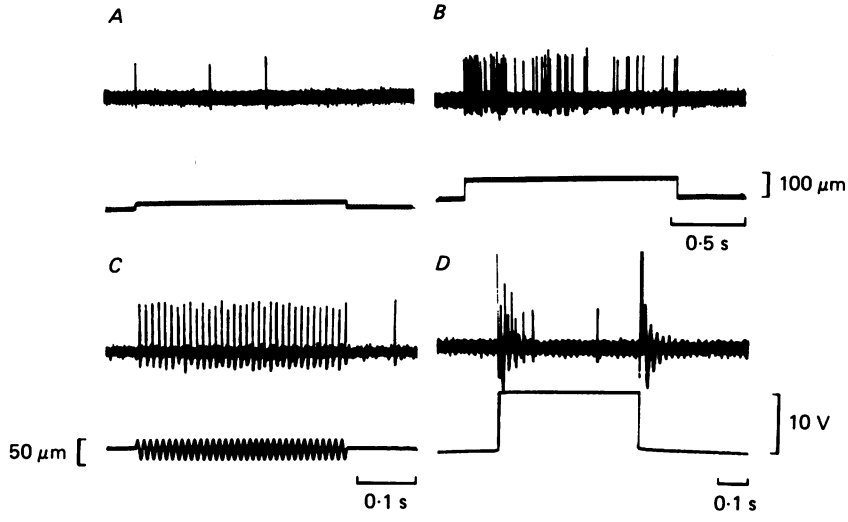


Fig. 9. Mechanoreceptor with intermediate adaptation rate. In each of the four panels the upper trace shows the afferent discharge, the lower trace in *A*, *B* and *C*, the monitored movement. In *D* the lower trace shows the square-wave voltage pulse used to stimulate this receptor electrically (7 V). In *C* the receptor responds 1:1 to 100 Hz sinusoidal vibration, 30 μm peak to peak amplitude. *A* shows a response at threshold (25 μm) indentation of skin; *B* shows a maximal response (80 μm). The 100 μm and 0·5 s calibration pulses to the left of *B* apply to both *A* and *B*.

responded weakly to vibration, giving a 1:1 response at 100 Hz and becoming intermittent at higher frequencies. Figure 9*D* shows the response of this receptor to electrical stimulation across the skin surface. On this occasion it was necessary to use 7 V to elicit a response. Immediately following the stimulus artifact a total of seven impulses were initiated. The first of these was used for an approximate estimation of conduction velocity. It was necessary to stimulate with up to 10 V to elicit impulses from mechanoreceptors using electric shocks, whereas by comparison the threshold of electroreceptors was 10–20 mV. A second receptor classified as intermediate in its adaptation rate had a much higher mechanical threshold (110 μm) and again adapted to silence within 2 s during maintained indentation of the skin. This receptor had a considerable vibration sensitivity, responding to mechanical oscillations at frequencies up to 600 Hz.

Three receptors classified as vibration receptors all responded to maintained skin indentation with one impulse during the on-phase and another impulse during the off-phase. All three were able to respond with one impulse per displacement of the vibrator up to frequencies of 600 Hz. At higher frequencies (700 Hz) this became 1:2 while beyond 800 Hz the receptor remained unresponsive. For two of the units the vibration amplitude required for a 1:1 response was measured over a range of frequencies and was at its lowest at 150–250 Hz and increased progressively at higher frequencies. Frequencies less than 70 Hz were not systematically tested. The receptive fields of all three receptors appeared to be quite localized but because of the high vibration sensitivity it was difficult to define their borders with any precision.

Structural correlates of mechanoreceptors

Attention in the present series of experiments was particularly directed at the newly discovered electroreceptors and attempts to correlate structure with function for the mechanoreceptors were unsuccessful. This was so despite the visible surface appearance of the push rods, which are almost certainly mechanoreceptors.

Conduction velocity

For seven units, including two mechanoreceptors and five electroreceptors, an attempt was made to estimate conduction velocity. The stimulating cathode was placed over the centre of the receptive field and stimulus strength increased until an impulse at fixed latency could be regularly elicited. The latencies for the two mechanoreceptors were both 1·4 ms and the estimated conduction distances of 30 and 34 mm give conduction velocity values of 21 and 24 m s^{-1} respectively. The situation for the electroreceptors was more complicated since the possibility existed that there was a synaptic interruption between the receptor cell and the afferent fibre (see Discussion). In any case quite short latencies were measured, 1·2–1·7 ms, to give estimates of conduction velocity of 19, 19, 20, 23 and 24 m s^{-1}. These may well be substantial underestimates if a synaptic delay of say, 0·5 ms should have been subtracted from the latency.

DISCUSSION

Electroreceptors

Our experiments together with those of Scheich *et al.* (1986) firmly establish the presence of electroreceptors in the bill of the platypus. Electroreceptors were

insensitive to mechanical stimuli, and showed a much greater sensitivity to electrical stimuli than mechanoreceptors, by more than two orders of magnitude. Electroreceptors had a resting discharge and responded to a steadily maintained depolarization, identifying them as tonic receptors resembling the ampullary receptors of fishes (Bullock, 1982). However, they do not fit neatly into the existing classification since, unlike other ampullary receptors, they also respond to rapidly changing potentials. Rays and sharks show feeding behaviour when exposed to slow cyclical potential fluctuations up to a frequency of 8 Hz and rays will respond to strong dipole currents up to 32 Hz (Kalmijn, 1974). The platypus receptors, on the other hand, responded to triangular potential fluctuations at a frequency of 150 Hz, well outside the range of the elasmobranch receptors.

One point of concern is that the thresholds for the platypus receptors were 20 mV or more. This compares with a value of 0·1–1 mV obtained for fresh-water fishes (Szamier & Bennett, 1980; Teeter & Bennett, 1981). Behavioural experiments on fresh-water fish suggest a sensitivity range of 15–120 μV cm^{-1} (Kalmijn, 1974). It has been suggested (Murray, 1974) that the ten to 100 times lower thresholds measured in behavioural experiments, compared with values obtained in electrophysiological experiments, can be accounted for by spatial summation of similar responses in many parallel channels. On these grounds a measured threshold of 20 mV is not incompatible with a behavioural threshold of less than 200 μV cm^{-1} (Scheich *et al.* 1986).

Excitation of the receptors required that the stimulating cathode was placed over the point of maximum sensitivity. Anodal stimulation reduced the discharge. The same polarity configuration is found in elasmobranchs, while in teleost fishes anodal stimulation excites the receptors. It is relatively easy to explain the mode of stimulation when the outside of the receptor, the ampullary lumen, is made positive by the stimulus but less straightforward when the stimulus for excitation is lumen-negative.

Electroreceptors in elasmobranch fish consist of a jelly-filled canal of varying length, open at one end to the surface while at the other end, the ampulla, lie receptor cells (neuromasts), their apical, luminal faces showing specializations such as microvilli or a kinocilium. Adjacent receptor cells are electrically insulated from one another and their luminal faces are separated from the basal faces by tight junctions with supporting cells. Transmitter release, to excite underlying nerve endings occurs at the basal faces. When the adequate stimulus for excitation is lumen-positive, that is, with the stimulating anode over the mouth of the canal, current flows inwards into the cell from the canal tending to hyperpolarize the luminal membrane and outwards through the basal face tending to depolarize it. The depolarization results in transmitter release and excitation of nerve endings. When the stimulus polarity for excitation is the reverse, the depolarization of the luminal segment of the receptor cell is thought to be propagated either passively or by a regenerative response to the basal segment triggering transmitter release (Bennett & Clusin, 1979).

The problem with a comparison between the platypus receptors and all other electroreceptors is that descriptions of the structure of the platypus electroreceptors suggest quite a different organization. The present results establish that at least one of the three kinds of 'gland–duct receptors' described by Andres & von Düring (1984),

the large mucus-secreting innervated variety (mucus sensory gland) is an electroreceptor. The innervated mucus sensory glands have small protoplasmic protrusions of myelinated axons reaching through the presumed receptor epithelium to the lumen of the gland as fine filamentous processes (Plate 1*B*). While the mechanism of stimulus transduction remains a matter of pure speculation, it may simply be that a cathodal pulse applied to the mouth of the gland results in depolarization of the filamentous ends of the axon and the depolarization propagates passively to the myelinated portion of the axon to trigger an action potential at a node of Ranvier. The extensively folded axolemma in the terminal portion of the myelinated axon may provide a large surface area to facilitate electrogenic pumping of ions across an inherently unstable membrane. The membrane is presumably constantly depolarized to maintain a resting discharge of impulses. If the voltage stimulus does lead to direct excitation of the nerve membrane it would result in a very short stimulus–response latency, similar to that for phasic receptors of fish which have electrically coupled synapses (Bennett & Clusin, 1979). The latencies measured by us are consistent with such an interpretation. However, further speculation of this sort must wait for more direct evidence about events at the level of the receptor.

Another point concerning the question of maintenance of a resting discharge is that the axons supplying gland duct receptors are conspicuous by the presence within them of filamentous material, large numbers of mitochondria, and of vesicles (Plate 1*B*) (Bohringer, 1981; Andres & von Düring, 1984). Are these, together with the membrane foldings, specializations necessary for maintaing high levels of resting activity? And what is the function of the non-innervated glands that alternate with innervated glands? Perhaps they simply help to keep the bill moist when it is out of the water and therefore maintain a low-resistance pathway between receptors and the skin surface.

Since both the electrophysiology and the behavioural experiments indicate that the platypus is able to respond to steady electric fields as well as rapid potential transients, some speculation is possible about the significance this may have for the animal in its natural environment. Most of the time at least, the platypus shuts its ears, eyes and nose during a dive. The eyes would be largely ineffective anyway since platypuses are known to frequent muddy streams (Barrett, 1941). The animal's ability to detect steady electric fields may well allow it to discern contours and obstacles on the stream bottom and the location of the river bank as a result of streaming potentials (Kalmijn, 1974). It is known that fish develop steady potentials across their body surface and that these potentials may be modulated by respiratory movements. Crustaceans have smaller surface potentials, but these become very large if the animal is slightly injured (Kalmijn, 1974). Platypuses are known to feed on small crustaceans, fishes and amphibians. It is possible that the prey is detected simply by its maintained electric field and in the case of the fresh-water shrimp, muscle activity associated with the tail flick (Scheich *et al.* 1986, and Fig. 7). Because of the wide dynamic range of the electroreceptors, the platypus may be able to 'see' a surprising amount of detail in its murky world as it fossicks about on the stream bottom. The characteristic horizontal sweeping movements of the bill are perhaps necessary to maintain voltage gradients in an optimal sensitivity range. The striking

alignment of receptors along the bill in parallel lines may also be of significance here (Fig. 1 and see Andres & von Düring, 1984). The platypus is the only known semiaquatic mammal with electroreceptors. How well these function out of the water and the possible significance they may have for the animal when the bill is in the air (see Barrett, 1941, p. 54) must remain, for the time being, a matter of speculation.

The evidence suggests that amongst sub-mammalian vertebrates electroreceptors evolved independently at least three times (Bullock, 1982). But on all occasions the ancestral receptor derived from the acoustico-lateralis system (Murray, 1974). Discovery of electroreceptors in the platypus adds a new dimension to this scheme. Not only is the platypus the only mammal known to be equipped with electroreceptors but the only known vertebrate with such receptors supplied by the trigeminal nerve. Furthermore it appears that the structural design of the platypus receptors is unique. The platypus therefore provides one further example of independent evolution of this receptor system. Such a conclusion underlines the proposition that monotremes have evolved a long way from their ancestral stock to become highly specialized in the adaptation to their particular environment. 'The premammalian characters of the platypuses only identify the antiquity of their lineage as a separate branch of the mammalian tree...in opposition to the myth of primitivity: (it really is) a superbly well-designed creature for a particular, and unusual mode of life' (Gould, 1985).

An important question which remains for the future is whether the echidna, too, has electroreceptors. Certainly gland duct receptors, even if simpler in structure, appear to exist in the skin of the echidna (Andres & von Düring, 1984). During the study of receptors in the echidna snout by Iggo *et al.* (1985) evidence for the presence of electroreceptors was not specifically sought. It therefore remains a real possibility that electroreceptors are found in both kinds of living monotremes.

Mechanoreceptors

The data on mechanoreceptors are somewhat scanty and will be dealt with only briefly. What can be said is that there are probably at least three different kinds of mechanoreceptors in the skin of the bill. The slowly adapting receptors with their irregular discharge resemble in some respects the cutaneous type I receptors found in the skin of other mammals (Iggo & Muir, 1969). However, the platypus receptors appear to be much more sensitive to dynamic stimuli than type I receptors and in this respect resemble more the slowly adapting mechanoreceptors with a regular discharge (SA type II, Chambers, Andres, von Düring & Iggo, 1972). A possible receptor structure which could give rise to these responses in the platypus is the Merkel cell receptor lying at the base of each push rod (Andres & von Düring, 1984).

The vibration receptors described in this study resemble in many respects similar responses recorded from the skin of the echidna snout (Iggo *et al.* 1985). A curious feature of the responses which deserves further attention in the future is that the optimal frequency of 150–250 Hz appears to be rather lower than that of other mammalian vibration receptors (Hunt, 1961), avian receptors (Dorward & McIntyre, 1971) and reptilian receptors (Proske, 1969). However, it is of interest that vibration receptors from the interosseus region of the hindlimb of the kangaroo were less

rapidly adapting than Pacinian corpuscles of other mammals (Gregory, McIntyre & Proske, 1986). Nevertheless the optimal frequency of the marsupial receptors was still 300 Hz or more.

Little can be said of the two receptors with intermediate adaptation rates. One of these which showed a relatively high vibration sensitivity may well have been a vibration receptor which during steady skin indentation responded to extraneous vibration transmitted through the stimulating probe. The second receptor had a very high threshold and responses were too feeble to permit a detailed study.

The question central to a discussion of mechanoreceptors in the platypus bill is, what is the function of the 'push rod'? Both Bohringer (1981) and Andres & von Düring (1984) considered it had a mechanoreceptive function. Attempts in the present investigation to correlate structure and function with respect to the mechanoreceptors were unfruitful and the role of the push rods must await further study.

The animals were obtained while one of us (U.P.) held a current permit from the Victorian Department of Fisheries and Wildlife (Permit No. 85–51). The participation of Ainsley Iggo in the experiments was made possible by a travel grant from the Carnegie Trust for the Universities of Scotland.

REFERENCES

ANDRES, K.-H. & VON DÜRING, M. (1973). Morphology of cutaneous receptors. In *Handbook of Sensory Physiology*, vol. 2, ed. IGGO, A., pp. 3–28. Berlin, New York: Springer.

ANDRES, K.-H. & VON DÜRING, M. (1984). The platypus bill. A structural and functional model of a pattern-like arrangement of different cutaneous sensory receptors. In *Sensory Receptor Mechanisms*, ed. HAMMANN, W. & IGGO, A., pp. 81–89. Singapore: World Scientific.

BARRETT, C. (1941). *The Platypus*. Melbourne: Robertson & Mullens.

BENNETT, M. V. L. & CLUSIN, W. T. (1979). Transduction at electroreceptors: origins of sensitivity. In *Membrane Transduction Mechanisms*, ed. CONE, R. A. & DOWLING, J. E., pp. 91–116. New York: Raven Press.

BOHRINGER, R. C. (1981). Cutaneous receptors in the bill of the platypus (*Ornithorhynchus anatinus*). *Australian Mammal* **4**, 93–105.

BOHRINGER, R. C. & ROWE, M. J. (1977). The organization of the sensory and motor areas of cerebral cortex in the platypus (*Ornithorhynchus anatinus*). *Journal of Comparative Neurology* **174**, 1–14.

BULLOCK, T. H. (1982). Electroreception. *Annual Review of Neuroscience* **5**, 121–170.

CHAMBERS, M. R., ANDRES, K.-H., VON DÜRING, M. & IGGO, A. (1972). The structure and function of the slowly adapting type II mechanoreceptor in hairy skin. *Quarterly Journal of Experimental Physiology* **57**, 417–445.

DORWARD, P. K. & MCINTYRE, A. K. (1971). Responses of vibration-sensitive receptors in the interosseus region of the duck's hind limb. *Journal of Physiology* **219**, 77–87.

GOULD, S. J. (1985). To be a platypus. *Natural History* **8**, 10–15.

GREGORY, J. E., IGGO, A., MCINTYRE, A. K. & PROSKE, U. (1986*a*). Sensory receptor in the bill of the platypus. *Journal of Physiology* **382**, 120*P*.

GREGORY, J. E., IGGO, A., MCINTYRE, A. K. & PROSKE, U. (1986*b*). Electroreceptor in platypus. *Proceedings of the Australian Physiological and Pharmacological Society* **17**, 144*P*.

GREGORY, J. E., IGGO, A., MCINTYRE, A. K. & PROSKE, U. (1987). Electroreceptors in the platypus. *Nature* **326**, 386–387.

GREGORY, J. E., MCINTYRE, A. K. & PROSKE, U. (1986). Vibration-evoked responses from lamellated corpuscles in the legs of kangaroos. *Experimental Brain Research* **62**, 648–653.

HUNT, C. C. (1961). On the nature of vibration receptors in the hind-limb of the cat. *Journal of Physiology* **155**, 175–186.

IGGO, A., MCINTYRE, A. K. & PROSKE, U. (1985). Responses of mechanoreceptors and thermoreceptors in skin of the snout of the echidna *Tachyglossus aculeatus*. *Proceedings of the Royal Society* B **223**, 261–277.

IGGO, A. & MUIR, A. R. (1969). The structure and function of a slowly adapting touch corpuscle in hairy skin. *Journal of Physiology* **200**, 763–796.

KALMIJN, A. J. (1974). The detection of electric fields from inanimate and animate sources other than electric organs. In *Handbook of Sensory Physiology*, vol. III/3, *Electroreceptors and other Specialised Receptors in Lower Vertebrates*, ed. FESSARD, A., pp. 147–200. Berlin: Springer.

LOEWENSTEIN, W. R. & ISHIKO, N. (1962). Sodium chloride sensitivity and electro-chemical effects in a Lorenzinian ampulla. *Nature* **194**, 292–294.

MÜNZ, H., CLASS, B. & FRITSCH, B. (1984). Electroreceptive and mechanoreceptive units in the lateral line of the axolotl *Amblystoma mexicanum*. *Journal of Comparative Physiology* A **154**, 33–44.

MURRAY, R. W. (1974). The Ampullae of Lorenzini. In *Handbook of Sensory Physiology*, vol. III/3, *Electroreceptors and other Specialised Receptors in Lower Vertebrates*, ed. FESSARD, A., pp. 125–146. Berlin: Springer.

PROSKE, U. (1969). Vibration sensitive mechanoreceptors in snake skin. *Experimental Neurology* **23**, 187–194.

QUILLIAM, T. A. (1966). The mole's sensory apparatus. *Journal of Zoology*, **149**, 76–88.

SCHEICH, H., LANGNER, G., TIDEMMAN, C., COLES, R. B. & GUPPY, A. (1986). Electroreception and electrolocation in platypus. *Nature* **319**, 401–402.

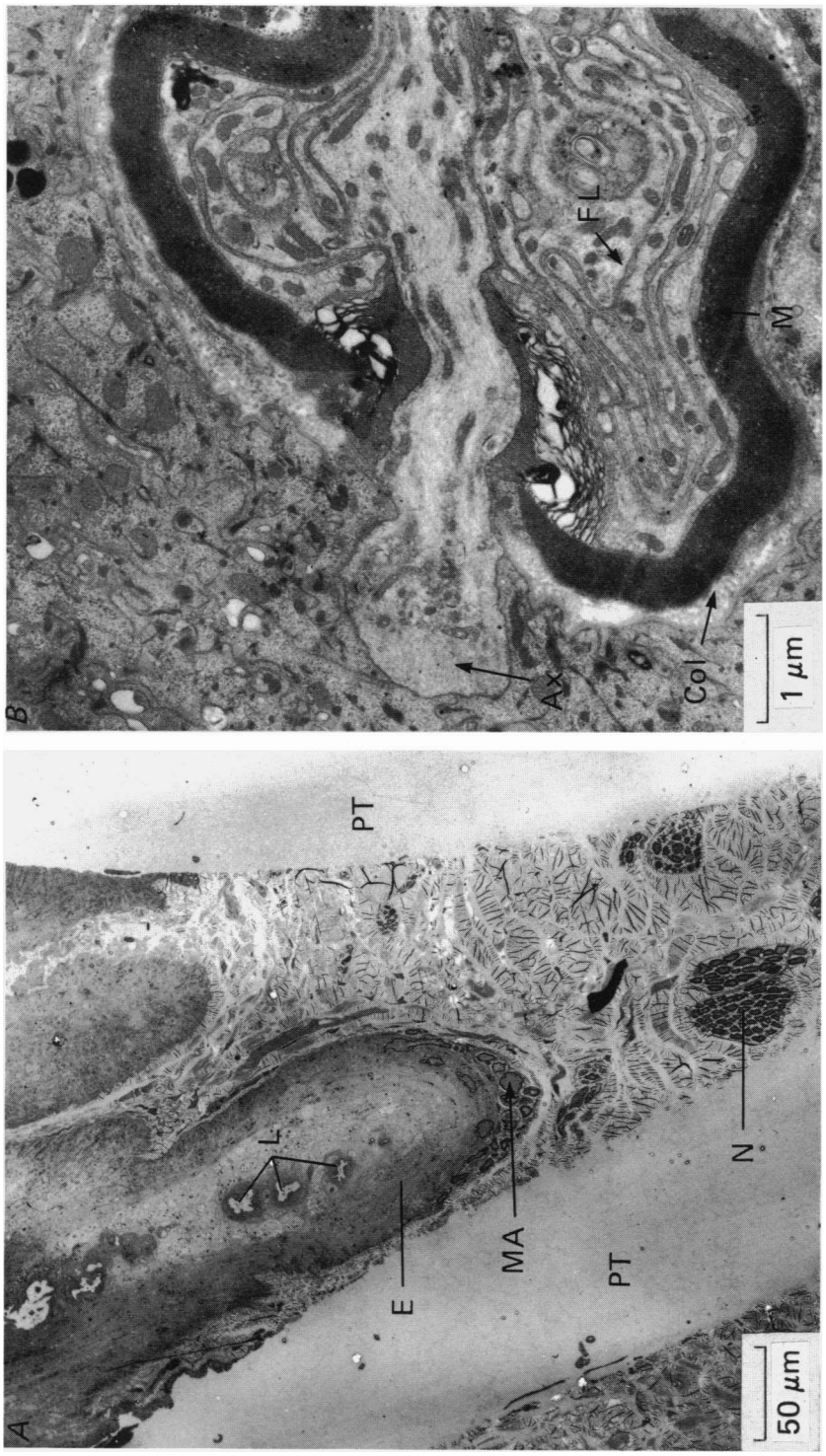
SZAMIER, R. B. & BENNETT, M. V. L. (1980). Ampullary electroreceptors in the freshwater ray *Potamotrygon*. *Journal of Comparative Physiology* **138**, 225–230.

TEETER, J. H. & BENNETT, M. V. L. (1981). Synaptic transmission in the ampullary electroreceptor of the transparent catfish *Kryptoperus*. *Journal of Comparative Physiology* **142**, 371–377.

TEETER, J. H., SZAMIER, R. B. & BENNETT, M. V. L. (1980). Ampullary electroreceptors in the sturgeon *Scaphirhynchus platyrhynchus* (Rafinesque). *Journal of Comparative Physiology* **138**, 213–223.

EXPLANATION OF PLATE

A, semi-thin sections of marked skin of platypus bill. The tracks made by the entomological pins, inserted while recording from the afferent fibres of the anaesthetized animal can be seen as clear spaces (PT) at each side of the micrograph. Between them lies the tissue of the epidermal duct (ED) of mucus sensory gland. The lumen (L) of the duct is visible at several places. In the outer wall at the epidermal base of the duct there is a group of myelinated axons (MA) and slightly more deeply at N in the dermis there is a small nerve, containing myelinated axons. *B*, high-power view of one of the axons lying embedded in the epidermal duct of the mucus sensory gland. M, myelin sheath; Ax, tongue of axon protruding into the deeper cellular layers of the gland duct. Notice the large number of mitochondria within the axoplasm. FL, extensively folded axon membrane produced by invagination of innermost layers of myelin. Immediately outside the axon are many collagen fibres (Col) providing structural support.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