Histological observations on presumed electroreceptors and mechanoreceptors in the beak skin of the long-beaked echidna, *Zaglossus bruijnii*

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SUMMARY

Sensory receptors in the rostral portion of the beak skin of a single specimen of the rare long-beaked echidna, *Zaglossus bruijnii*, are described. Mucous glands which have been modified to accommodate sensory innervation, similar to those seen in *Ornithorhynchus*, are found in the rostral 2 cm of the beak skin, anterior to the maxillofacial foramen, at a density of approximately 12/mm². The papillary epidermal portion of the gland ducts are walled by concentric layers of keratinocytes, and each duct is innervated by 10–15 myelinated nerve terminals. The mucous gland receptors in *Zaglossus* are intermediate in structure between those of *Ornithorhynchus* and *Tachyglossus*, but are similar enough to the former to suggest that electroreception may play a major role in the sensory experience of *Zaglossus*. Push-rod mechanoreceptors also occur throughout the same region of beak skin, and appear similar to those described for *Tachyglossus*.

1. INTRODUCTION

The New Guinea echidna, *Zaglossus bruijnii*, commonly known as the long-beaked echidna, is one of the three extant species of monotremes. *Zaglossus* is easily distinguished from the other echidna species, *Tachyglossus*, as its external anatomy differs quite dramatically. Of particular interest for the present study is the difference in the morphology of the echidna beak. *Zaglossus* has a beak which is approximately twice the length of that of *Tachyglossus*, and this beak is curved in contrast to the straight beak of *Tachyglossus*. The present geographical range of *Zaglossus* is limited to the high (1200–2800 m altitude) humid montane forests of Irian Jaya through to eastern Papua (Van Deusen & George 1969). The 1990 IUCN Red List of Threatened Animals, and appendix 2 of the CITES, list *Zaglossus* as vulnerable. Thornback & Jenkins (1982) have calculated that at the time there were approximately 300 000 *Zaglossus* remaining in the montane forest of New Guinea.

Only one previous study of the histological appearance of sensory receptors in the beak skin of *Zaglossus* has been undertaken (Kolmer 1925). Kolmer reported very briefly that there were many glands in the beak skin, noted to have many ‘Lamellenkörperchen’ (lamellae bodies) surrounding the outward channel of the duct, and located at the level of the junction of the dermis and epidermis. From

a series of recent studies (Scheich *et al.* 1986), we now know such glands are in fact electroreceptors, and that the configuration that Kolmer described is typical of electroreceptors in other monotremes (Andres & von Düring 1984, 1988; Andres *et al.* 1991; Gregory *et al.* 1987, 1988; 1989; Manger & Hughes 1992; Krubitzer *et al.* 1995; Manger & Pettigrew 1995, 1996; Manger *et al.* 1995). Secondly, Kolmer reported rod-like structures throughout the skin, akin to those found in the platypus bill skin as reported by Poulton (1884). Again, from more recent studies (see above list) in both *Ornithorhynchus* and *Tachyglossus*, we know these structures to be push-rod mechanoreceptors. Kolmer noted that these push-rods had only been described in the monotremes and birds (for a recent review see Gottschaldt 1985), however, other ultrastructural studies have revealed that they are also present in the moles, family Talpidae (Eimer 1871; Armstrong & Quilliam 1961; Halata 1972; Catania 1995) and analogues are found in the snout skin of the hedgehog (Pac & Malinovsky 1981) and the rhinarial skin of the opossum (Munger 1965).

The electroreceptive system of *Ornithorhynchus* is far more complex than that of *Tachyglossus*, showing a range of specializations not seen in the latter (Manger & Hughes 1992; Manger & Pettigrew 1995, 1996; Manger *et al.* 1995). It has been suggested that the electroreceptive system in *Tachyglossus* is evolutionarily redundant, hence its rudimentary appearance (Manger & Hughes 1992).

In the light of recent studies of monotreme electroreceptivity, a reinvestigation of the presumptive electro-

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Figure 1. Photomicrographs of four different views of the presumed electroreceptor in the beak skin of *Zaglossus*, stained with toluidine blue. (a) A transverse section through the epidermal extent of an electroreceptor, demonstrating the innervation at the papillary region of the receptor which forms a nerve cuff (nc). The pore (p) at the surface of the epidermis is also seen; b, c, and d refer to the level of the sections in three associated photomicrographs. (b) A tangential section through the electroreceptor at the level of the nerve cuff. The duct (D) is seen in the centre of the concentric rings of keratinocytes, and the stratification of these keratinocytes is also seen. Four to five cells make up the periluminal layer (p), a series of 5–7 flattened keratinocytes make up the dense layer (d), and 2–3 cells constitute the germinative layer (g). The large bulbous portions of 15 electrosensory nerve terminals (arrows) are seen in the germinative layer. (c) A tangential section through an electroreceptor at a depth where the normal epidermal pegs end. The stratification of the layers is more obvious in this section than in the previous one. (d) A tangential section again closer to the surface than the previous two, at a level approximately equal to the top of the epidermal pegs. At this level all three layers have proliferated in numbers of cells, and the dense layer (d) has begun its transformation into the equivalent of the non-desquamating layer of the stratum corneum. Scale bars: (a) = 100 μ m, (b)–(d) = 50 μ m.

receptors in *Zaglossus*, as described by Kolmer (1925), is warranted, and the data obtained from this reinvestigation are presented here. The differences in diet, environment, and evolutionary position of

Zaglossus as compared with the other monotremes, allow us to speculate that perhaps *Zaglossus*, as a terrestrial animal, may indeed have some use for an electrical sense.

Figure 2. Three photomicrographs of the push-rod mechanoreceptors found in the bill skin of *Zaglossus*. (a) and (b) show transverse sections through the extent of the push-rod (R) and the innervating axons (ia) which supply the three types of mechanoreceptors associated with the push-rod. In both sections the rod is seen to be shorter than the length of the epidermal pegs, a feature typical of the monotremes. The standard configuration of keratinocytes within the rods is seen, and the fusing of the superficial portion of the rod to the surrounding epidermis is also shown. Some portions of the central and peripheral vesicle chains are also evident in the rod. (c) This photomicrograph shows two Paciniform corpuscles (arrows) amongst the innervating axons in the dermis below the rod (R). The loose outer layers and the tighter inner layers of the corpuscles can be seen. Scale bars: (a) and (b) = 100 μ m, (c) = 50 μ m.

2. MATERIALS AND METHODS

Histology of small samples of beak skin from only one specimen of *Zaglossus* is described in this paper. The skin was obtained from a specimen which died at Taronga Park Zoo in Sydney, Australia, and which was passed onto the Australian Museum. Shortly following death the carcass was placed in a freezer, and three months later was perfused with 10% formalin. During this freezing, fixing and handling of the specimen, the beak was broken in several places, and the

rostral 1 cm of the beak was crushed and discarded. However, enough skin posterior to the crushed region, located slightly superiorly to the corner of the mouth, was sufficiently preserved to enable histological examination. Due to the extended time between death, freezing and fixation, the quality of the material is not of the highest standard. Enough histological detail remains, however, to provide a brief description of the presumed electroreceptors and other sensory structures in the beak skin.

The skin specimens were sectioned in two planes, either

tangential to the skin surface, or orthogonal to the skin surface. This afforded us two views of the receptors, and also allowed examination of the innervation of the receptors in two planes. Specimens were embedded in glycol methacrylate, sectioned at 3 μm , and stained with toluidine blue.

3. RESULTS

Two types of sensory receptor complexes were identified histologically within the beak skin of *Zaglossus*. Because of the similarity of the receptors in *Zaglossus* to those of *Ornithorhynchus* and *Tachyglossus* they will, for convenience, be referred to as electroreceptors and push-rod mechanoreceptors.

(a) *Electroreceptor complexes*

The electroreceptor complex shows the typical configuration of the monotreme electroreceptor. This consists of a large dermal gland, which secretes a seromucous substance, and is connected to the surface of the skin via a duct which traverses the epidermis. The innervation associated with the gland is located below the level of the epidermal pegs, at the papillary region of the duct (see figure 1). At this region myelinated axons form a neural cuff surrounding the duct.

The neural cuff of *Zaglossus* differs in some respects from that of both the other monotremes. Between 10 and 15 myelinated axons penetrate the epidermis surrounding the duct of the gland, approximately 120 μm below the level of the adjacent epidermal pegs (see figure 1*a, b*), at which point they form the large bulbous portion of the electrosensory nerve terminal. These had an average diameter of 10 μm (range 8–12 μm) (see figure 1*b*). Other features of the nerve terminals such as smaller bulbous portions, terminal

filaments and circumferential arbors, described for *Ornithorhynchus* (Manger *et al.* 1995), were not apparent in the present preparations from *Zaglossus*.

The pores of these glands were easily seen under a dissecting microscope at the surface of the epidermis, and had a diameter of approximately 100 μm . The cells of the epidermis surrounding the duct showed an arrangement approximating to that found in *Ornithorhynchus*. Surrounding the lumen of the epidermal portion of the gland duct in *Zaglossus* were 4–5 loosely packed epidermal cells, the periluminal layer. Encircling this layer was a layer of 5–7 tightly packed, flattened keratinocytes, constituting the dense layer, and surrounding the dense layer was a layer of 2–3 cells forming the germinative layer (see figure 1*b, c, d*) (terminology from Manger *et al.* 1995).

Although the exact number and distribution of these presumed electroreceptors could not be determined from the present specimen some details may be important. The sensory glands were limited in their distribution, and only occurred rostral to the maxillofacial (Murray 1984) foramen (also known as the infra-orbital foramen (Andres *et al.* 1991)), where it can be presumed the trigeminal innervation of these receptors arises (see figure 4), probably in a similar manner to that described for *Tachyglossus* (Andres *et al.* 1991). The maxillofacial foramen in *Zaglossus* is located approximately 2 cm from the rostral tip of the beak. The region of skin studied showed a density of 12 electroreceptors/ mm^2 (see figure 3), and from equivalent studies in *Tachyglossus*, it can be assumed that the numbers of receptors would increase towards the rostral pole of the bill. It was obvious from the specimen that there were many more electroreceptors in *Zaglossus* than *Tachyglossus*. All glands examined in

Figure 3. Photomicrograph of a tangential section through a large region of the epidermis showing the complementary distribution of the electroreceptors (e) and the push-rod mechanoreceptors (r). The densities of the two receptors in this section are the same, indicating that these two receptors probably have a similar number and distribution, and that there may be some functional interaction. Scale bar = 250 μm .

Figure 4. Photographs of the skull of *Zaglossus* demonstrating the foramen from which trigeminal axons innervating the sensory receptors in the rostral portion of the beak skin arise. (a) Dorsal view of the skull showing the upper foramina (arrows). (b) Ventral view of the skull showing the lower foramina (infra-orbital or maxillofacial, see text) (arrows). (c) Lateral view of the skull showing the relative position of both the foramen, the lower foramen being positioned slightly caudal to the upper foramen. (d) A higher power view of the rostral portion of the beak with the upper and lower foramina clearly seen (large arrows), as well as some more caudal foramina (small arrows) from which axons innervating more caudal portions of the beak arise. The sensory receptors described in this study are limited in their distribution anteriorly to the two major foramina which are located approximately 2 cm from the rostral pole of the beak. Scale bars (a)–(c) = 2 cm, (d) = 1 cm.

Zaglossus were innervated. While *Zaglossus* has more electroreceptors than *Tachyglossus* there are far fewer than in *Ornithorhynchus*.

(b) *Push-rod mechanoreceptor complex*

A push-rod mechanoreceptor has been described in both *Ornithorhynchus* (Manger & Pettigrew 1996) and

Tachyglossus (Manger & Hughes 1992), and appears to be a complex formed by the unification of three types of mechanoreceptive nerve endings with a transducer rod of epidermal cells which may act to increase tactile sensitivity.

In *Zaglossus*, the length of the rod, from the surface of the epidermis to its base is 250 μm , and the rod has an average diameter of 45–50 μm (see figure 2a, b).

Table 1. *Comparison of the anatomical features of the electroreceptors and push-rod mechanoreceptors between the three species of monotremes*

(Note first that the electroreceptors of *Zaglossus* are intermediate but closer to those of *Ornithorhynchus*, suggesting that they play a major role in the sensory experience of *Zaglossus*. Secondly, the push-rod mechanoreceptors of *Zaglossus* are closer to those of *Tachyglossus*. Finally, this table discloses the gaps in our present knowledge of the anatomy of the sensory receptors found in the beak/bill skin of monotremes, and so provides a basis for future ultrastructural studies. The information for this table is derived from the present study and those of Andres & von Düring (1984, 1988), Andres *et al.* (1991), Manger & Hughes (1992), Manger *et al.* (1995) and Manger & Pettigrew (1996).)

	<i>Ornithorhynchus</i>	<i>Zaglossus</i>	<i>Tachyglossus</i>
electroreceptor features			
dermal mucous gland	present	present	present
papillary nerve cuff	complex	extensive	simple
terminals per duct	16	10–15	3–5
dia. large bulb	16 µm	10 µm	5 µm
small bulb	present	?	absent
terminal filaments	present	?	present
circumferential arbors	present	?	absent
pore diameter	100 µm	100 µm	30–50 µm
duct cell lamination	complex	extensive	vague
cells in periluminal layer	2–3	4–5	2–3
cells in dense zone	10–15	5–7	2–3
cells in germinative layer	1–2	2–3	1–2
number of electroreceptors	40 000	?3000?	100
density	30/mm ² , in stripes	12/mm ²	?, but less than <i>Zaglossus</i>
% mucous glands innervated	100 %, in stripes, 60 % of total	100 %	25 %
distribution	in stripes over entire bill skin	rostral 2 cm of beak skin	rostral 1 cm of beak skin
epidermal portion length	600 µm	450 µm	300 µm
epidermal portion diameter	150 µm	120 µm	70 µm
push-rod mechanoreceptor features			
3 types of nerve terminals	present	present	present
number of vesicle chains/receptor	50	?	13
number of Merkel cells/receptor	12	?	12
number of Paciniform corpuscles/receptor	3–6	?	2
rod length	400 µm	250 µm	300 µm
rod diameter	70 µm	50 µm	50 µm
attachment to superficial epidermis	absent	present	present
other structures in bill skin	sensory serous glands non-sensory mucous glands	?	non-sensory mucous gland, possible serous gland

Myelinated axons arising from a nearby dermal plexus were seen to innervate three types of mechanosensory nerve endings: vesicle chain receptors within the rod, Merkel cells at the base of the rod, and Paciniform corpuscles in the dermis below the rod (see figure 2*c*). The poor condition of the tissue prevents us from providing exact details as to the number and sizes of these neural structures.

The push-rods in *Zaglossus* are shorter in length than the surrounding epidermal pegs, a feature common to

echidna push-rods, and so are easily distinguished from the surrounding tissue. At the superficial portion of the rod, the layers of keratinocytes are seen to be fused to the surrounding epidermal layers (see figure 2*a, b*).

At the region examined the push-rods exhibited the same density as that of the electroreceptors. It is probable that their distribution does not extend caudal to the maxillofacial foramen, and that towards the rostral pole of the beak there would be a significant rise in the number of push-rods.

4. DISCUSSION

The present description of sensory receptors in the beak skin of *Zaglossus* is the first since the electroreceptive capabilities of the monotremes were discovered (Scheich *et al.* 1986). The histological details of the sensory receptors found in *Zaglossus* are compared with what has been reported for the other monotremes in table 1. In particular the number and size of electroreceptive nerve terminals per sensory gland, the actual number of glands, the distribution of the glands, and the arrangement of keratinocytes within the glands demonstrate that the electroreceptors in the beak skin of *Zaglossus* are much closer to those of *Ornithorhynchus* than to those of *Tachyglossus*.

These details that vary across the monotremes are important, as they can be related to the processing of electrical information (Manger *et al.* 1995). They all indicate that *Zaglossus* has an electroreceptive system that is conceivably functional. The habitat of *Zaglossus* is probably more conducive to the reception of electrical signals than the varied habitat of *Tachyglossus* (Griffiths 1978). *Zaglossus* locates its major prey item, earthworms, in the floor of the humid montane rainforests, by probing with its beak and scratching with its claws in the litter of the forest (Griffiths 1978). The movements of the earthworms through this litter may create an electrical signal that is detectable by *Zaglossus*; measurements of the sensitivity of *Zaglossus* electroreceptors, the electrical fields a moving earthworm would produce, and the soil conductivity are required before this assumption can be confirmed.

If all three extant species of monotremes have an electroreceptive system it is highly likely that the stem monotreme was also electrosensitive. Although monotreme phylogeny and evolution is an unresolved issue (Augee 1984), present understanding of the evolution of monotremes is as follows. The first monotremes were of the Ornithorhynchid lineage, and diverged from other mammals sometime between 130 and 160 million years ago (Mya) (Archer *et al.* 1985). There appears to be no radiation of the Ornithorhynchid lineage until the late Cretaceous (60 Mya) when the Tachyglossid line diverged (Westerman & Edwards 1992). If at the time of the echidna radiation, the platypus's were already water dwellers with an elaborate electrosensory system, which seems likely, then the echidnas would have had a sensory system which would be of apparently little use on land. However, at this period, the Australian continent was quite tropical in nature, consisting mainly of rainforest, similar to the modern habitat of *Zaglossus* (Archer 1984; Murray 1984). The wet rainforest floor may provide the opportunity for the use of a reduced electroreceptive system, possibly similar to that found in *Zaglossus*. As the continent dried out (Archer 1984; Murray 1984), *Tachyglossus* appears to have adapted to the arid conditions, and virtually completely lost the use of its electroreceptive system. *Zaglossus* has become more limited in its range, restricted to that habitat which would support its lifestyle (Murray 1984).

As for the push-rods, they appear so similar in *Zaglossus* and *Tachyglossus* that it is highly likely they

have the same function, which previous physiological experimentation in *Tachyglossus* suggests is a mechano-receptive function (Gregory *et al.* 1989).

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