Complex Sensory Corpuscles in the Upper Jaw of Horsfield's Tortoise (*Testudo horsfieldii*)

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Abstract

The sensory corpuscles of *Testudo horsfieldii* in the skin of the upper lip and face were studied with light and electron microscopy. The sensory corpuscles were situated under epidermis; in the corium and also between the upper jaw bone tissues in the rostral part of oral cavity. The skin sensory corpuscles with a ramified inner core were grouped in clusters. Within the corpuscle there were several simple inner cores embedded within a common superficial capsule. The complex corpuscles have a novel structure in comparison to what has been described for sensory nerve endings in turtle. The complex sensory corpuscles probably function as mechanoreceptors important for monitoring the movement of the keratinized ridges and the most rostral part of the upper jaw, the rhamphotheci.

Mechanoreceptors, reptiles, Testudines, electron microscopy, lamellated encapsulated receptors

The upper jaw region has rich innervations containing many different receptors. For this reason, it is an important source of information. The innervation of the rostral part in head includes skin sensory receptors and sensory receptors of the oral cavity. In contrast to mammals, less attention has been paid to the structure of sensory corpuscles in the reptilian jaws. This study contributes to the understanding of innervations in the tortoise face and describes a novel type of encapsulated sensory corpuscles.

The reptilian sensory corpuscles were first described in the skin and oral cavity by several authors in the 19th century (Leydig 1872; Cartier 1872; Leidy 1878; Merkel 1880) and later the studies have continued up to now (Hulanicka 1913; Aota 1940; Hin-Ching and Maneely 1962; Dingler 1964; Miller and Kasahara 1967; Siminoff and Kruger 1968; Proske 1969; Landman 1975; Soares 2002). However, they are mainly focused on crocodilian or snake sensory corpuscle structure.

The caiman face is covered with many small pigmented domes of integumentary sensory organs filled with lamellated receptors (von Düring 1973). These receptors are now named on the basis of functional observations as the dome pressure receptors (Soares 2002), and they can detect disruptions in the surface of surrounding water.

A detailed study of receptors in the upper jaw of *Boa constrictor* showed six different types of tissue receptors in their scales (von Düring 1974). Three types are located intraepithelially (radiant heat receptor, bubble receptor, receptor axons) and three types are laid in the corium (lamellated free receptors, branched lanceolate terminals, touch papillae). Physiological examination in the same species *Boa contrictor* (Hensel 1975) revealed the presence of warm receptors in their labial region, while no cold-sensitive fibres were

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found. Nishida et al. (2000) discovered sensory papillae located along the teeth rows of the snake (*Elaphe quadrivirgata*). Each sensory papilla is comprised of a taste bud, free nerve endings in the epithelium, Meissner-like corpuscles, branched coiled terminals and lamellated corpuscles in the connective tissue.

Dingler (1964) found simple sensory corpuscles in *Egernia cunnighami* (Scincidae). Miller and Kasahara (1967) reported on the innervations of lizard skin and Proske (1969) on those of the snake *Pseudechis porphyriacus*. Landmann and Halata (1980) examined the labial epidermis of the lizard (*Podarcis siculus*) and found Merkel cells and nerve endings there. Burnasheva (1965) described corpuscles in the ducts of the Harderian gland in lizards and tortoises.

Less attention has been given to the turtle sensory system. Hin-Ching and Maneely (1962) described branched coiled terminals of the dermis in the soft-shelled turtle *Pelodiscus sinensis* (syn. *Trionyx sinensis*). A different type of encapsulated corpuscles were formed in the joint capsules of the tortoise *Testudo graeca* and *Emys orbicularis*: spray-like ramified encapsulated corpuscles with an inner core (Páč 1968, 1975). Von Düring and Miller (1979) mentioned the presence of encapsulated lamellated receptors in *Testudo graeca* without detailed documentation and description. In contrast to other reptilian groups, the upper jaw of cheloniids is covered by keratinous beak – the rhamphotheca (Wyneken 2003). Tortoises are edentulous, therefore the sharp edges of the rhamphotheca take the functional place of teeth. Considering the different external morphology and function of the upper jaw, we have posed the question whether the innervation of the tortoise lip is adapted to the changed functional requirements.

Materials and Methods

The sensory corpuscles with the inner core were studied with light and transmission electron microscopy (TEM). Five upper jaws were collected from adult tortoise – *Testudo horsfieldii* Gray, 1844. Each of these patients was euthanized due to the clinical status related to chronic liver disease and/or chronic renal failure. The intramuscular injection of ketamine/xylazine was followed with T61 into the dorsal tail vein (vena coccygea dorsalis).

Two heads were fixed in 10% formaldehyde for light microscopy. After dehydration the material was embedded in paraffin, serial histological sections were prepared (5 μm) and stained with either Gömöri stain, haematoxylin-eosin or Alcian blue.

Three upper jaws for electron microscopy were fixed in 4% glutaraldehyde following 2% solution of OsO_4. After dehydration the material was embedded in Durcupan (ACM Fluka). Semithin and ultrathin sections were made on Tesla BS ultramicrotome. The ultrathin sections were treated with the water solution of uranylacetate and lead citrate to increase contrast. They were examined and photographed on Tesla BS 500 electron microscope.

Results

Sensory corpuscles are situated under epidermis in the upper jaw. The tortoise jaws do not contain teeth but around the margins there are sharp ledges formed by a thick layer of keratinized epidermis (Plate I, Fig. 1, Plate II, Fig. 2a). Under the epidermis there is a thin zone of dermis composed of several layers of dense collagenous connective tissue distinctly alternating in direction. Many chromatocytes are situated near epidermis (Plate II, Fig. 2a, Fig. 2b). Dermal papillae are absent. The sensory corpuscles are not only in the corium but also between the upper jaw bone tissues in the rostral part of the oral cavity (Fig. 2b). The corpuscle diameter varies from 60 to 150 μm.

The ultrastructural studies showed a novel arrangement that has not been described previously for turtle sensory organs. The corpuscles in the upper jaw have a ramified inner core and they are grouped together in clusters of several axons. Their inner structure is more complicated than the structure of simple sensory corpuscles. There are some simple inner cores located inside the corpuscle. The complex structure – all simple inner cores is embedded inside the common superficial capsule.

Each simple inner core is formed by a centrally located axon (its dendritic zone) and by few cells of inner core arranged around it (Plate II, Fig. 2c,d). The myelinated axon

enters into the corpuscle (Plate II, Fig. 2e). After passing through the capsule, the myelin sheath comes to an end (near the inner core border). Cells of the inner core are modified Schwann cells (Plate II, Fig. 2c,d,f). The individual inner cores are not separated by the parts of capsular lamellar cells. There are structures of boundary space (Fig. 2c,e) filled with collagenous microfibrills and individual fibrocytes situated among the individual (neighbouring) inner cores. The whole corpuscle is bounded with a simple lamellous capsule (Plate II, Fig. 2g,h). The capsule is formed by flat lamellar cells from two to four layers. These capsular cells are of epineural type.

Disscusion

The sensory nerve endings of *Testudo horsfieldii* belong to the IIIb class using the classification of Malinovský and Páč (1982). These nerve endings are called the sensory corpuscles and their terminals are in the relation to Schwann cells. These cells form the lamellar inner core.

Until now, only one publication reported turtle sensory corpuscles in the orofacial region. Hin-Ching and Maneely (1962) described nerve endings in *Pelodiscus sinensis*. In the snout area they demonstrated very different types of nerve endings (only simple receptors) from our finding in *Testudo horsfieldii*. von Düring and Miller (1979) in their review of reptilian sensory nerve endings mentioned the presence of encapsulated lamellated receptors in *Testudo graeca* as unpublished observations. Unfortunately, they did not provide any photographical documentation or close description of their observations, and the location of the receptors was not characterized. Thus, it is difficult to compare our findings but we assume the presence of similar corpuscles also in other chelonians.

The upper and lower jaws of cheloniids are covered by the rhamphotheca (Wyneken 2003) and the shape of these keratinous beaks varies with diet (Schumacher 1973; Ruckdeschel et al. 2000). Tortoise sensory corpuscles lay near the base of these keratinous beaks. We suggest that the corpuscles function to monitor beak movement similarly to Herbst corpuscles and Ruffini corpuscles in birds. Although the tortoise sensory corpuscles are located in the same part of orofacial region as the corpuscles in birds, there are two major differences in the ultrastructure. The tortoise corpuscles are grouped in clusters with a ramified inner core, which we can never see in Herbst corpuscles (Malinovský and Páč 1980). The Ruffini corpuscles are also arranged in groups of up to ten corpuscles (Halata and Grim 1993) in the beak of Japanese quail (*Coturnix japonica*), similarly to tortoise corpuscles, but each corpuscle is surrounded by an incomplete fibrous capsule.

Comparing the tortoise corpuscles to other reptilian species, Nishida et al. (2000) discovered lamellated corpuscles in the snake oral cavity that lacked a capsule. Similar arrangement to our tortoise corpuscles was described in the caiman (von Düring 1973; von Düring and Miller 1979). These sensory corpuscles also have a looser lamellation with capsule space. In comparison to *Caiman*, *Natrix* and *Varanus* (von Düring 1973) where the lamellated receptors are associated with papillae, our tortoise corpuscles do not seem associated with a specific surface structure of the face. Caiman receptors are more similar to our findings but only rarely two receptor axons may run parallel inside one single capsule space. In tortoise receptors, two to five axons can run together within a common capsule. While caiman sensory corpuscles are components of the touch papillae and this area is covered by a thin skin layer (von Düring 1973), tortoise sensory corpuscles occur in a deeper dermis layer between bones and have no contact with the surface. Therefore the tortoise corpuscles may function quite differently from the caiman corpuscles.

Does the similarity of tortoise and bird corpuscles reflect a functional adaptation for beak movement monitoring? Or do they reflect a conserved developmental programme of facial structures in turtles and birds compared to scaled reptiles? This question requires further examination of turtle species with a different arrangement of facial structures, e.g. the

leatherback sea turtle *Dermochelys coriacea* that lacks a distinct rhamphotheca on either jaw (Wyneken 2003).

Some of the recent reports discussed the phylogenetic position of the turtles (Hill 2005). Molecular findings of mitochondrial genomes indicated that turtles are not a basal lineage among the anapsid reptiles (Zardoya and Meyer 1998; Cao et al. 2000; Janke et al. 2001), and the anapsids seem to be paraphyletic themselves (Rieppel 1999). Mindell et al. (1999) found a sister relationship between turtles and a bird/crocodilian clade. Caspers et al. (1996) indicated that turtles branched off from the amniote tree after the mammals. Turtles have a partially developed secondary palate and a more complicated structure of sensory corpuscles in the upper jaw compared to scaled reptiles. Molecular data support the new morphological findings that turtles are more derived than early studies proposed (Carroll 1988). Our finding of complex sensory corpuscles in the *Testudo horsfieldii* suggests the necessity of further study of sensory corpuscles in other reptiles in order to determine where the complex sensory corpuscles originate phylogenetically. Malinovský and Páč (1982) suggested that amphibians and reptiles only have simple corpuscles, yet our recent findings of sensory corpuscles with a ramified inner core in tortoise showed that in reptiles also complex corpuscles are present. Since simple lamellated receptors were described in amphibians (Bolgarskij 1964; von Düring and Seiler 1974), complex sensory corpuscles probably appeared first in reptiles.

Složitá smyslová tělíska v horní čelisti *Testudo horsfieldii*

Smyslová tělíska, která se nacházejí v kůži horní čelisti u želvy, *Testudo horsfieldii,* byla studována pomocí světelného a transmisního elektronového mikroskopu. Tělíska jsou umístěna pod epidermis, ve škáře a rovněž mezi kostními lamelami horní čelisti v rostrální části ústní dutiny. Tato komplexní nervová zakončení mají odlišnou strukturu ve strovnání s dosud popsanými tělísky u želv. Tělíska vytváří shluky, z nichž každé obsahuje několik zón označovaných jako vnitřní jádra, která jsou obaleny společnou povrchovou pochvou. Jednotlivá vnitřní jádra mají centrálně umístěný axon a modifikované Schwannovy buňky ho obklopují. Tato složitá smyslová tělíska pravděpodobně působí jako mechanoreceptory, které jsou nezbytné pro monitorování pohybu zrohovělých okrajů čelistí, přičemž jeho ostré okraje slouží jako funkční náhrada dentice u želv.

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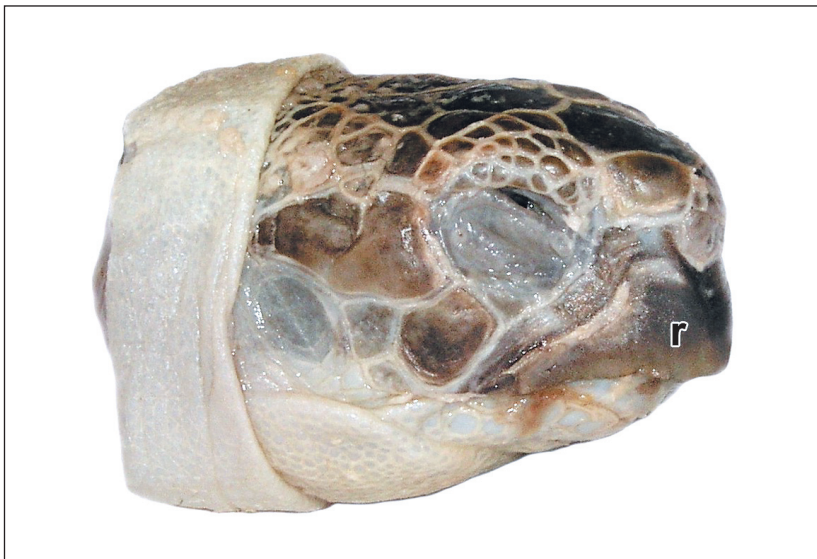
Plate I
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Fig. 1. The head of a Horsfield's tortoise, r – rhamphotheca
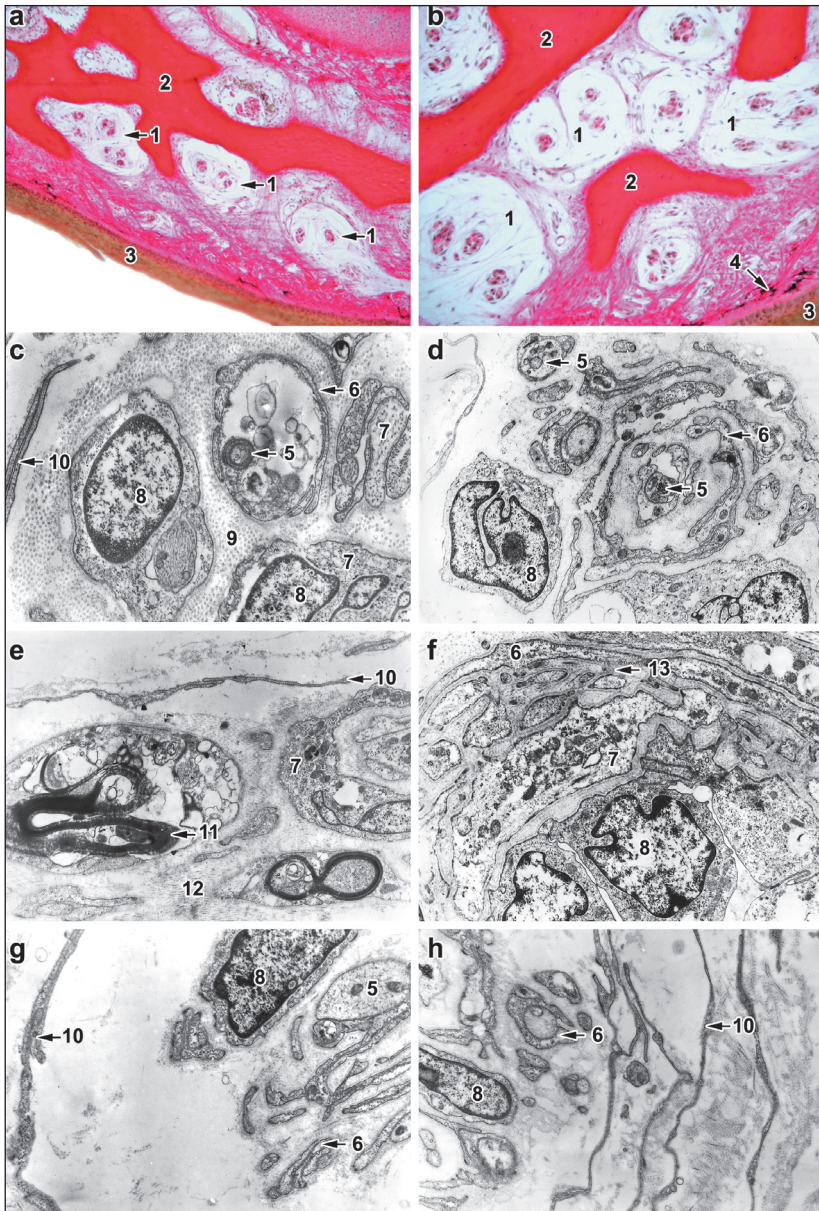
Plate II

Fig. 2. Transversal section of the upper jaw. a, b: Hematoxylin-eosin, c-h: TEM.
1 – sensory corpuscles, 2 – bone tissue, 3 – epidermis, 4 – corium with chromatocytes, 5 – axon (sensory nerve terminal), 6 – inner core cell lamellae around nerve terminal, 7 – inner core cell, 8 – nucleus of inner core cell, 9 – boundary space filled with collagen microfibrils, 10 – capsular lamellae, 11 – axon with myelin sheath entering the inner core, 12 – boundary space, 13 – inner core space. Magnification: a - × 100, b – × 200, c-h – ×12.000