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The 'goatee' of goatfish: innervation of taste buds in the barbels and their representation in the brain

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Goatfish use a pair of large chin barbels to probe the sea bottom to detect buried prey. The barbels are studded with taste buds but little else is known about the neural organization of this system. We found that the taste buds of the barbel are innervated in a strict orthogonal fashion. The barbel is innervated by a main nerve trunk running in the core of the barbel. A longitudinal nerve bundle originates from the main trunk and, after running a short distance distally, divides into two circumferential nerve bundles (CNB) extending respectively, medially and laterally around the barbel. Approximately 15 CNBs innervate each 1 mm length of barbel. At each transverse level, the CNB innervates two clusters of taste buds, each containing 14 end-organs. The primary taste centre in the brain is similarly extraordinary. The sensory inputs from the barbel terminate in a derived dorsal facial lobe, which has a highly convoluted surface forming a multitude of tubercles. Electrophysiological mapping experiments show that the entire barbel is somatotopically represented in a recurved elongate tubular fashion within the dorsal facial lobe.

Keywords: taste; taste buds; barbel; innervation; somatotopy; goatfish

1. INTRODUCTION

Goatfish are a diverse group of tropical and temperate benthic marine fishes. They have a large pair of barbels (like a goatee) extending downward from their lower jaw and use these barbels to probe the loose substrate materials in search of food. The barbels are quite rigid and are moved rapidly to both probe and stir the substrate. Once a potential prey item is encountered, the fish strikes at the particular location on the bottom and takes the food item into its mouth. The fish's ability to locate food is not hindered by removal of the olfactory or visual senses since the barbels are studded with numerous taste buds (Sato 1938; Holland 1978).

Other fishes are known to have elaborate, specialized taste systems, e.g. catfish and carp (Morita & Finger 1985*a*,*b*). In general, taste buds situated on the outside of the body of the fish are innervated by branches of the facial nerve, which terminates centrally in the rostral end of the viscero-sensory column of the medulla. By contrast, intraoral or pharyngeal taste buds are innervated by the glossopharyngeal and vagus nerves, which terminate more caudally in the same medullary column. Catfish, with numerous taste buds on their 'whiskers', or barbels, have a large, somatotopically organized facial lobe (Marui *et al.* 1988; Hayama & Caprio 1989; Kiyohara & Caprio 1996; Kiyohara *et al.* 1996). By contrast, carp and goldfish, with a specialized intraoral food-sorting apparatus have a large, vagal lobe (Morita *et al.* 1983; Morita & Finger 1985*a*; Sibbing *et al.* 1986).

Previous studies have revealed that goatfish exhibit an unusual elaboration of the facial lobe, but the exact nature of the structure has not been elucidated (Kiyohara 1988; Barry & Norton 1989). The facial lobe in goatfish appears as a cauliflower-like protrusion from the dorsal surface of the medulla. This is strikingly different from the large facial lobes of other fishes. This report describes the unique specializations of both the peripheral taste apparatus and its unusual representation within the central nervous system.

2. MATERIAL AND METHODS

(a) *Materials*

Two species of goatfish, *Parupeneus trifasciatus* (figure 1*a*) and *P. pleurotaenia*, weighing 50–120 g, were used in this study. The goatfish were caught at the coast, near the Tropical Biosphere Research Centre, University of the Ryukyus Sesoko Station, Okinawa, Japan where the majority of the work was undertaken. For the data reported here, no differences were noted between these species.

(b) *Morphological experiments*

The carbocyanine dye 1,1′diocadecyl-3, 3,3′, 3′-tetramethylindocarbocyanine perchlorate (DiI) was used to trace the peripheral distribution of barbel nerves and applied as described by Finger & Böttger (1990). The fishes were anaesthetized with dilute (approx. 1 : 10 000) tricaine methansulphonate (MS 222) and perfused through the conus arteriosus by 4% paraformaldehyde in 0.1 M phosphate buffer. The barbels, with the barbel nerve intact, were removed from the fishes and cut into several pieces. Approximately 1–2 mm of barbel nerve were exposed in each cut piece at its proximal part and these pieces were embedded in 3% agar. The stump of the barbel nerve was exposed by cutting the agar block with a razor. Small crystals of DiI were applied to the cut surface of the nerve and fresh agar was poured over the surface of the nerve to keep the DiI in place, and to prevent accidental migration of DiI to other regions of the barbel. The agar block was placed into the same fixative in a 37 °C oven for 10–90 days. In some cases, DiI was dissolved in one drop of 95% ethanol. This solution was sucked into the glass capillary and was allowed to dry. DiI flakes on the tip of the capillary were applied to a single taste bud under the dissecting microscope. After the diffusion period, the agar was removed from the pieces of barbel. The pieces were cut with a razor, in

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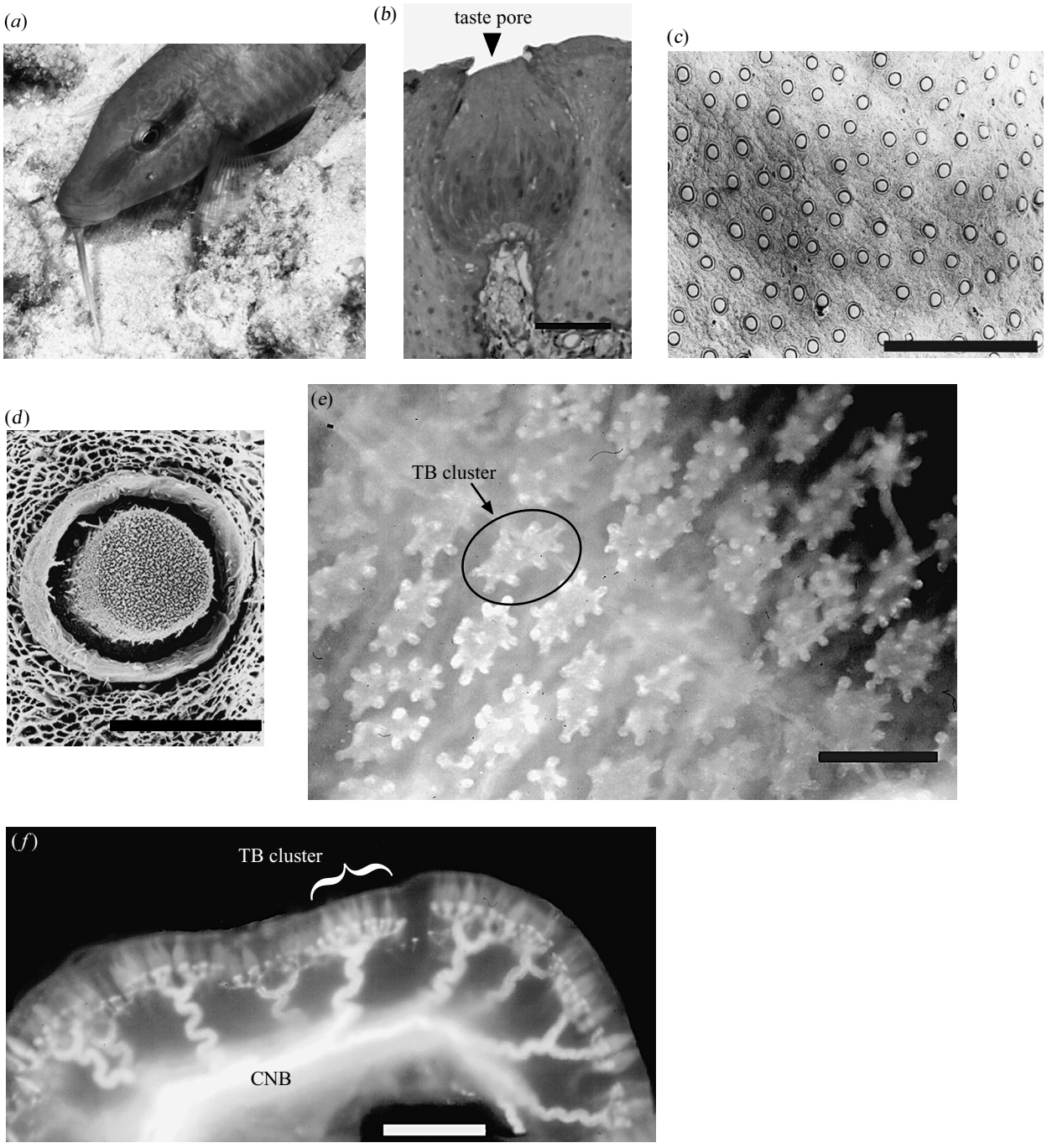

Figure 1. (*a*) The goatfish, *Parupeneus trifasciatus*, possesses a pair of elongate, stiff barbels as major sensory organs. The fishes use these barbels to probe the substrate in search of prey (photograph courtesy of Mr Y. Hirai). (*b*) Toluidine blue-stained cross-section through a barbel showing the typical structure of a taste bud. The taste pore is a broad opening in the epithelium by which taste substances gain access to the sensory tips of the cells of the taste bud (scale bar, 30 μm). (*c*) Scanning electron micrograph of a barbel showing that the many taste pores of taste buds are uniformly distributed across the entire surface of the barbel (scale bar, 230 μm). (*d*) Higher magnification scanning electron micrograph showing the apical processes of the taste cells extending outward through a single taste pore (scale bar, 25 μm). (*e*) Surface view of a barbel in which the nerve processes have been stained with the fluorescence tracer, DiI. The labelled nerve fibres branch to form stereotypically organized nerve terminals beneath the taste-bud clusters in the epithelium (scale bar, 500 μm; TB, taste bud). (*f*) Cross-section through a DiI-labelled barbel showing each taste-bud cluster to be innervated by a single radial nerve bundle emanating from the circumferential nerve branches (scale bar, 500 μm; TB, taste bud).

the longitudinal or transverse plane, and their surface, or cut surface, was viewed by an Olympus standard or Leica dissecting epifluorescence microscope with a rhodamine-filter.

The barbels and brains were also processed using routine histological techniques (Kitoh *et al.* 1987) to reveal their morphological organization.

(c) *Electrophysiological experiments*

The receptive field of neurons in the facial lobe was mapped by recording electrical activities of a multi-unit. The fishes were immobilized by Flaxedil and positioned in a Plexiglas container. The gills and oral cavity were perfused with aerated seawater. The dorsal surface of the head was anaesthetized by topical

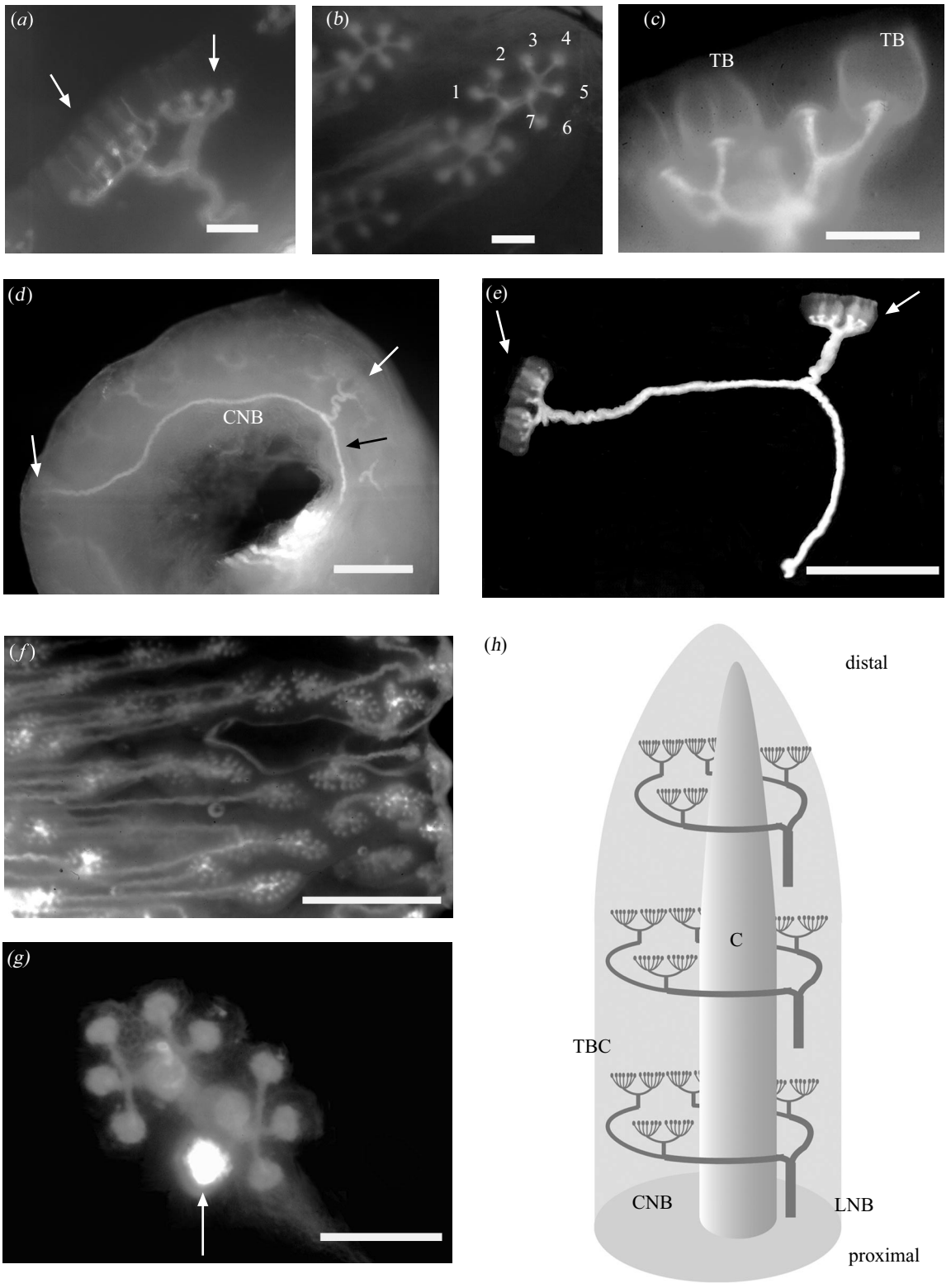

Figure 2. (*Caption overleaf.*)

application of 3% tetracaine. The skull was opened using a pair of rongerus and a dental drill. Electrical activity within the facial lobe was recorded extracellularly using glass microelectrodes (2–6 μm tip diameter; 1–5 MΩ impedance) filled with 3 M NaCl or 2% Pontamine sky blue in 0.5 M sodium acetate. The electrodes were inserted into the dorsal facial lobe to record the activity in response to tactile stimulation. The activity was amplified, integrated (time constant 0.5 s) and displayed on a pen recorder. A cathode-ray oscilloscope and audioamplifier also monitored the amplified activity. Receptive fields (RFs) for touch were located by touching the surface of a barbel with a fine glass probe (tip diameter of *ca.* 20 μm). Movement of the glass probe was controlled by the manipulator. Electrode placement was verified histologically by iontophoretic marking with the dye. After recording and dye marking, the fishes were perfused intracardially with marine teleost Ringer's solution, fol-

lowed by 4% paraformaldehyde in 0.1 M phosphate buffer (7.2 pH). The fixed brain was embedded in egg yolk and sectioned at 40 μm on a freezing microtome in the transverse plane.

3. RESULTS

(a) *Distribution of taste buds in the barbels*

Scanning electron micrographs of the barbel reveal densely packed, large taste buds with an interbud spacing of *ca*. 50 μm. Taste buds are distributed in the entire epithelium of the barbel along its entire length (figure 1*b–d*). In a species with a total length of 16 cm, the density of taste buds increases gradually from 150 mm^{-2} at the basal portion, to greater than 250 mm^{-2} at the tip of the barbel. At the basal region of the barbel, the taste bud-bearing epithelium is patchy, being intermingled with areas of non-sensory epithelium. Frequently, taste buds were distributed in groups of 12–16 sensory end-organs. In the middle and distal portions of the barbel, taste buds are distributed evenly across the entire epithelium.

The opening of the taste buds to the external environment, the so-called taste pore, is unusually large in goatfish. The taste pores in goatfish reach a distance of up to 20 μm compared with *ca*. 10 μm in the taste buds of other fishes (figure 1*b*,*c*). Another unusual feature of the taste buds in goatfish is the apparent absence of occasional thick apical microvilli (figure 1*c*), which are common in other fishes (Grover-Johnson & Farbman 1976; Reutter 1978; Kitoh *et al.* 1987; Royer & Kinnamon 1996).

(b) *Innervation of taste buds in the barbels*

When DiI is placed on the facial nerves at the base of the barbel, fluorescently labelled axons can be followed along the length of the barbel, and seen to ramify beneath the skin to innervate the base of the taste buds. Occasionally, elongate cells of the taste buds were labelled by transcellular diffusion of the dye (Finger & Böttger 1990; Kotrschal *et al.* 1993), but usually only the nerve plexus at the base of the taste bud was labelled. In these fishes, the nerve plexus forms a disk embracing the basal ends of the taste cells (figure 2*a–c*). When viewed from the surface, the taste buds are arranged in clusters, each receiving its own branch of the main barbel nerve trunks (figure 1*e*,*f*). Each cluster comprises two hemi-clusters, each usually containing seven taste buds (range of 5–8; figure 2*a–c*). The taste buds in a cluster cover an oval area of *ca*. 500 × 200 μm. This clustered pattern of innervation is not evident if the arrangement of taste pores is observed, cf. figure 1*d*. The innervation of taste buds in the barbel follows an orthogonal system. The principal barbel nerve runs longitudinally along the lateral edge of the cartilaginous core of the barbel. Fascicles leave the main trunk along the posterior margin of the core to form distally directed longitudinal bundles, which extend a short distance along the length of the barbel. Each longitudinal bundle gives rise to paired circumferential branches extending medially and laterally around the margins of the barbel to innervate the taste bud clusters at that proximal–distal level (figure 2*d*,*e*). Figure 2*f* shows deep and superficial focal planes from a single specimen to illustrate the circumferential branches and the overlying taste-bud clusters. The axons of each circumferential branch innervate two clusters of taste buds at that particular transverse level; therefore, when DiI is placed into a single taste bud, labelled fibres are present in all taste buds along the same circumferential branch (figure 2*g*).

Thus, one longitudinally running nerve branch (LNB) is a functional unit, originating from the main trunk and dividing into two circumferential nerve bundles (CNBs) extending respectively, medially and laterally around the barbel (figure 2*h*). Each CNB innervates two taste bud clusters in the epithelium. Since each taste bud cluster contains approximately 14 taste buds, each CNB innervates 28 taste buds. Thus, each longitudinal bundle innervates 56 taste buds located at a defined transverse level of the barbel. We can use this value to estimate the total number of taste buds on a barbel. There are, on average, 15 CNBs per 1 mm length of barbel. Using this value, we can estimate the number of LNBs, CNBs and taste buds in the barbel. For example, a 20 cm specimen of *P. trifasciatus* possesses 4 cm of barbel. In this barbel, there would be approximately 600 CNB and therefore 33 600 taste buds.

Electron microscopy of the nerve bundles at various regions of the barbel reveals that the LNBs and the CNBs each contain similar numbers of axons. Most of the LNBs consist of approximately 90 fibres with a diameter less than 2 μm; some LNBs contain these fine fibres plus some coarse fibres greater than 3 μm in diameter. These results

Figure 2. Innervation of taste buds in the barbels of goatfish. The nerves are filled with the fluorescent tracer DiI. (*a*) Lateral view of one cluster of nerve terminals. Note that one cluster consists of two hemi-clusters (arrows), which are innervated by the circumferential branch (scale bar, 100 μm). (*b*) Surface view of a cluster showing that each hemi-cluster consists of seven terminal arbours, one beneath each taste bud of the cluster (scale bar, 100 μm). (*c*) Side view of a hemi-cluster (scale bar, 100 μm; TB, taste bud). (*d*) A transversally cut barbel showing one circumferential branch (CNB, black arrow), which originated from a longitudinal bundle at the right side of the barbel to run around the central rod cartilage that was removed in this piece of barbel. This branch ends in two terminal branches (white arrows; scale bar, 1 mm). (*e*) One circumferential branch was dissected out from the above preparation (*d*) (scale bar, 1 mm). Note that this branch innervates two clusters of taste buds (white arrows). (*f*) Surface view of a longitudinally cut barbel showing many circumferential branches and underlying clusters. These branches vary in length but do not cross the centreline of the barbel to reach the other side. The long axis of the barbel is orientated vertically in this figure (scale bar, 1 mm). (*g*) Connectivity of a cluster of taste buds. DiI was applied to a single taste bud (white arrow) with the aid of a glass needle. Dye diffuses to all the adjacent taste buds within a cluster showing nerve fibres innervating one bud of a cluster branch to reach all other buds within the same cluster (scale bar, 200 μm). (*h*) Schematic representation of the pattern of innervation of taste buds in the barbel of goatfish. The longitudinal nerve branch (LNB) originating from the main nerve trunk sends a pair of circumferential branches medially and laterally around the margins of the barbel to innervate four taste-bud clusters (TBCs) at each proximal–distal level. Each cluster consists of 14 taste buds. Each longitudinal branch, which consists of approximately 90 fibres innervating 56 taste buds, is a functional unit. Each 1 mm length of barbel contains approximately 15 CNBs.

are consistent with those in which DiI was placed in a single taste bud; each nerve fibre of the circumferential branch innervates all taste buds innervated by the branch as a whole.

(c) *Morphology of the primary taste centre*

In goatfish, as in all other vertebrates, the gustatory fibres of the facial nerve enter the rostral medulla to terminate at the rostral end of the visceral sensory column. The rostral medulla of goatfish exhibits an elaborate facial lobe protruding dorsally from the floor of the fourth ventricle and extending beneath the caudally deflected cerebellum. When the cerebellum is removed, it is apparent that the facial lobe is not smooth but is marked by numerous tubercles (figure 3*a*,*b*). Sections of the facial lobe reveal that the lobe is divided into several major subdivisions, of which only the most dorsal exhibits the tubercular appearance (figure 3*c*). Histologically, the tubercles appear coarsely laminated with a superficial molecular layer, an intermediate layer of densely packed medium neurons and a deeper layer of elongate, larger neurons (figure 3*d*,*e*). Filling of various peripheral nerves shows that the barbel is represented in the dorsal, tubercular portion of the facial lobe while nerves innervating the rest of the face and head terminate in the ventral subdivision of the lobe (data not shown). Within the dorsal portion of the facial lobe, the primary afferent terminals end within the superficial, molecular layer of the lobe.

(d) *Somatotopical representation of barbels in the primary taste centre*

In order to better understand the nature of the tubercles of the dorsal facial lobe, we used microelectrodes to map the receptive fields of neurons situated in various areas of the facial lobe. The electrode was driven vertically throughout the facial lobe in a systematic grid of points projected onto the dorsal surface of the lobe. Because of the convoluted nature of the system of tubercles of the dorsal facial lobe, in any single dorso-ventral electrode penetration, the electrode tip was likely to pass from one tubercle into another. Thus, the observed discontinuities in the receptive fields of adjacent areas were not unexpected. Penetrations through the rostromedial portion of the facial lobe yielded receptive fields near the base of the barbel, whereas penetrations along the lateral edge of the rostral part of the lobe revealed fields near the distal tip of the barbel. There was, however, no smooth continuity of receptive fields between these areas. For example, as shown in figure 4*a*, moving the electrode *ca.* 500 μm laterally, from position 1B to position 1C at middle levels showed receptive fields moving from the base of the barbel to midway along its length without any intermediate representation. However, such intermediately situated receptive fields were evident at more caudal levels of the lobe (level 3; figure 4*a*). When all recordings are taken into account, despite apparent discontinuities at any particular antero-posterior level, a continuous, albeit convoluted representation of the barbel could be discerned (figure 4*b*). The organization is comparable with allowing a dangling strand of spaghetti to coil haphazardly upon itself as it is lowered onto a platter.

4. DISCUSSION

This report demonstrates the unique specialization of the barbel taste system in goatfish. In general, the increase in the number of taste buds leads to an enlargement of the primary taste centres, such as the facial lobe in catfish or vagal lobe in cyprinids. One striking morphological feature of the primary taste centre of the goatfish is a remarkable enlargement of the dorsal facial lobe. This enlargement can be due the enormous number of taste buds as well as their large size. The dorsal facial lobe seems to develop within a limited space between the medulla and cerebellum. As a result, the dorsal facial lobe has become highly convoluted to adequately accommodate the brain tissue in a limited space. The primary taste centres of fishes are somatotopically organized (Morita & Finger 1985*a*,*b*; Marui *et al.* 1988; Hayama & Caprio 1989; Kiyohara & Caprio 1996; Kiyohara *et al.* 1996). In the somatotopic map, each part of the taste epithelium is represented in proportion to its relative importance in taste perception (Kanwal & Finger 1992). In the catfish, the entire body surface, from lips to caudal fin, is sharply defined in the facial lobe and the barbels are represented in especially enlarged lobules extending rostrocaudally. In goatfish, the barbels are represented in an even larger, tubercular structure. The apparent tubercles of the goatfish dorsal facial lobe are actually recurved flexures in a convoluted continuous columnar representation of the barbel.

In general, the barbels of fishes function as both tactile and taste organs, and play an important role in searching for food items in the environment. Catfish are known as another representative group of barbelled fishes. They have numerous taste buds in the epidermis of barbels throughout their length, with an increase in density toward the tip of the barbel, as in the goatfish. However, the catfish and goatfish make a great contrast in the neural organization of their barbels as well as the distribution of taste buds. The taste buds of the barbels in catfish such as *Ictalurus punctatus*, *Arius felis* and *Plotosus lineatus* are more densely distributed along the most rostral surface of the barbel than on any other areas (Kiyohara & Caprio 1996; Sakata *et al.* 2001). The barbel nerves of *Plotosus* are distributed to form networks of hexagonal shape under the epidermis, and pairs of strands originate from the networks to innervate taste buds (Sakata *et al.* 2001). The maximum and minimum diameters for the hexagons range from 200–300 to 80–150 μm, respectively. The number of taste buds located on one network ranges from 40 to 100 μm. Therefore, it appears that the fibres in the barbels of catfish are organized to innervate pairs of taste buds on the lines of networks. Conversely, the goatfish has taste buds distributed evenly at a certain level of the epithelium of barbels (figure 1*c*,*e*,*f*). The taste buds are innervated in a strict orthogonal fashion; one longitudinally running nerve bundle innervates approximately 54 taste buds located on the epidermis at a certain level of longitudinal extent of the barbel (figure 2*h*). The number of circumferential bundles is *ca.* 15 mm^{-1}. Thus, in the catfish barbel, a taste fibre bundle or a functional unit carries information received from some area of the barbel surface, to which the bundle is distributed to form networks. By contrast, in the goatfish barbel, one longitudinally running nerve bundle or a functional unit carries information obtainable

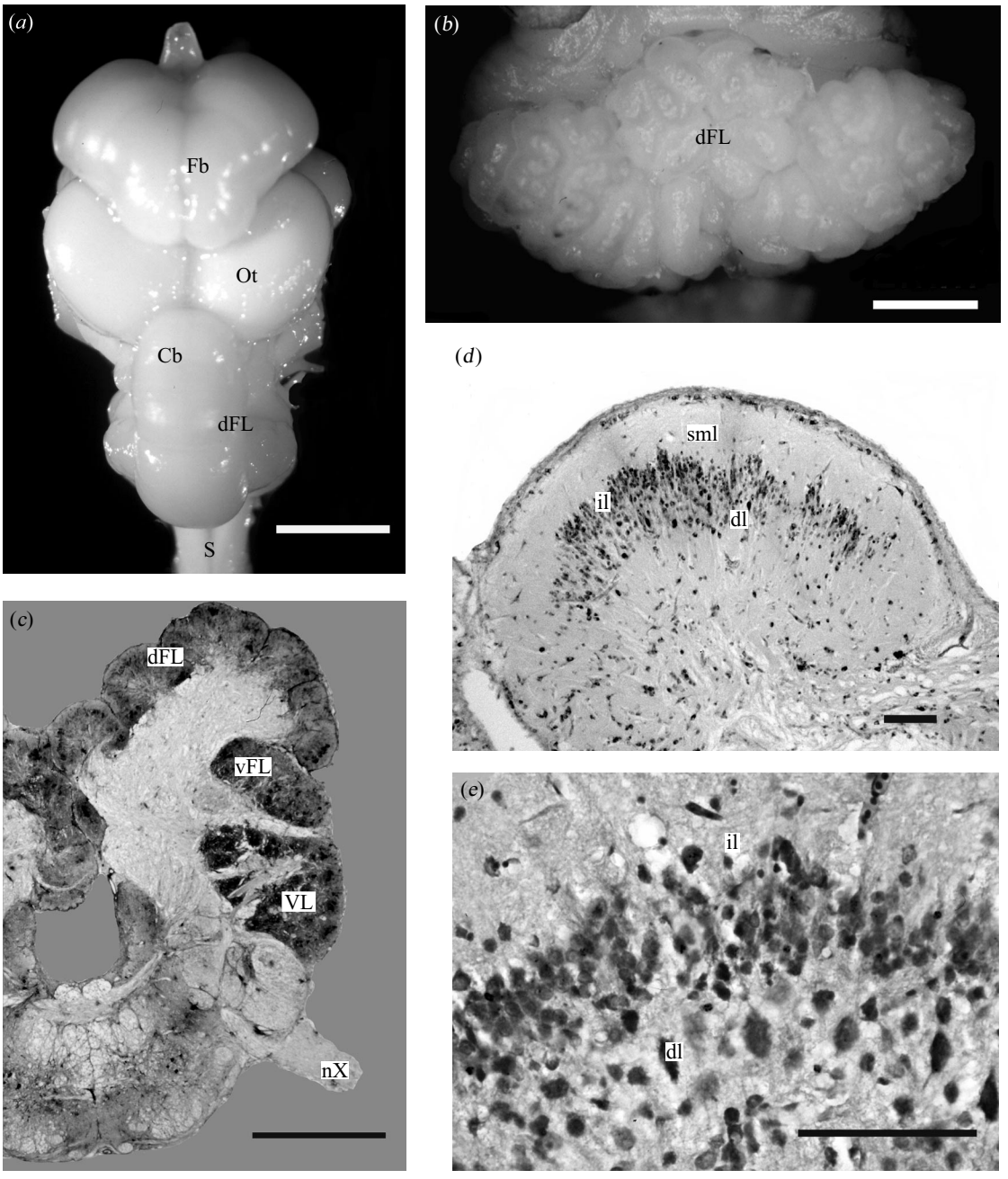

Figure 3. The primary taste centre of goatfish and cytoarchitecture of the facial lobe. (*a*) Dorsal view of the brain of *Parupeneus pleurotaenia*. Anterior is uppermost. Abbreviations: Fb, forebrain; Ot, optic tectum; Cb, cerebellum; dFL, dorsal division of facial lobe; S, spinal cord (scale bar, 0.5 cm). (*b*) Dorsal view of facial lobe. Membranes covering the facial lobe were removed to show the cauliflower-like surface of the facial lobe (scale bar, 2 mm). (*c*) Transverse section at a level in (*b*). Abbreviations: dFL, dorsal division of facial lobe; vFL, ventral division of facial lobe; VL, vagal lobe; nX, vagal nerve (scale bar, 2 mm). (*d*) Longitudinal section of a tubercle of the dorsal facial lobe showing lamination with a superficial molecular layer (sml), an intermediate layer of densely packed medium neurons (il) and a deeper layer of elongate, larger neurons (dl) (scale bar, 100 μm). (*e*) A higher magnification view of the cytoarchitecture of intermediate and deeper layers (scale bar, 100 μm).

from around the barbel surface at a certain level of longitudinal extent of the barbel.

In summary, the neural representation of the chin barbels of goatfish is highly unusual, both in terms of the orthogonal regularity of the peripheral innervation and in their representation in a tortuous, recurved somatotopy. The system appears to be specialized to permit the fish to accurately discern the depth of a prey object buried in the sand.

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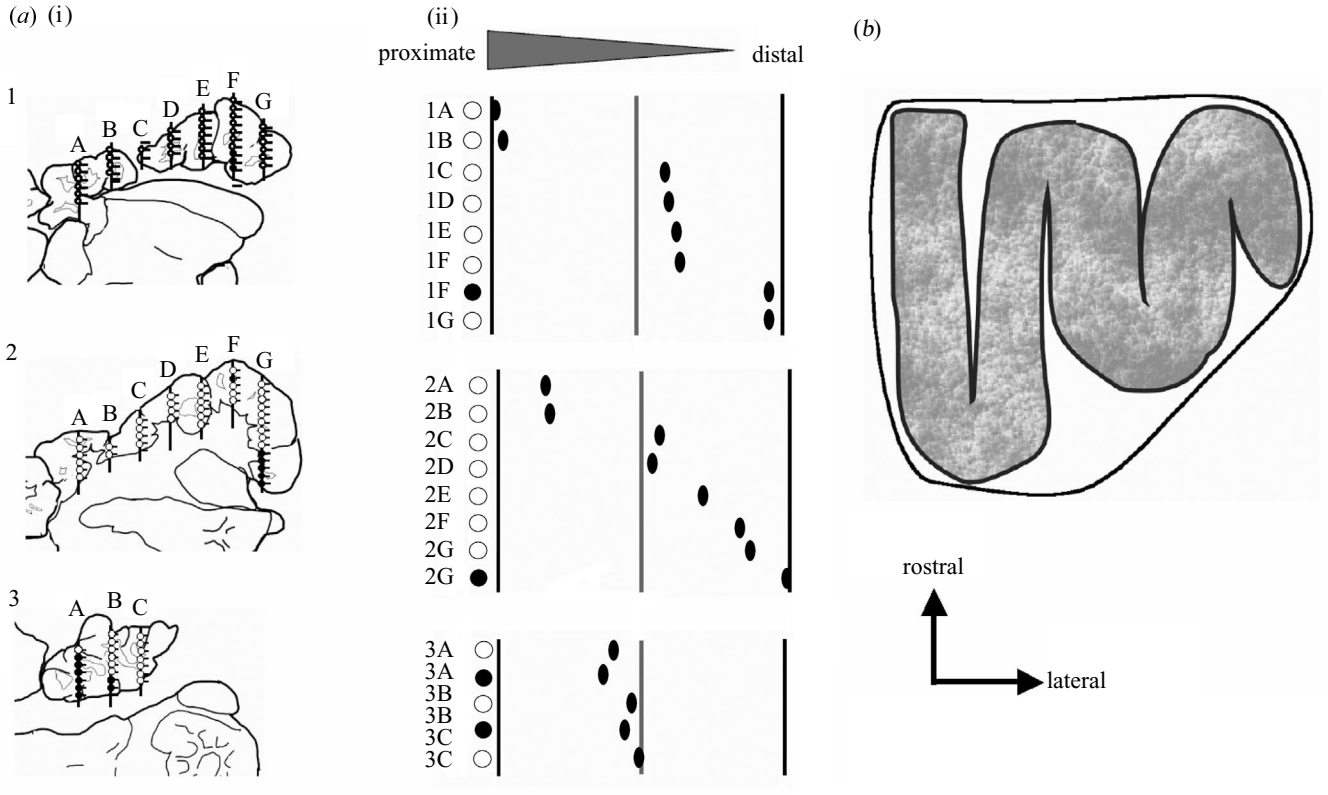

Figure 4. Somatotopic representation of the barbel in the dorsal facial lobe of goatfish. Multi-unit activity in response to mechanical stimulation was recorded in six transverse planes with 500 μm intervals. In each plane, a series of penetrations was made with the electrode being moved from the medial to lateral plane with 500 μm steps. For each penetration, the electrode was advanced downward in 100 μm steps. (*a*) (i) Examples of tactile receptive fields (RFs) for recording sites at three different transverse levels from rostral (1) to caudal (3). Intervals are 540 μm between 1 and 2, and 1080 μm between 2 and 3. (ii) RFs for the recording sites, which are indicated by open or solid circles along electrode tracks of each level, are shown as solid ovals located in a relative position of the barbel shown at the top. In 1, for example, the RF was located on the base of barbel for recording sites along the electrode track A. The RFs did not change as the electrode penetrated deeper in this track but the magnitude of the response changed considerably. The maximum response was always obtained when the electrode was positioned in the intermediate or deeper layer of the tubercle. In some tracks, such as 1F or 2G, RFs changed after the electrode reached a certain depth of the dorsal facial lobe. These changed sites were shown as solid circles and their RFs are shown separately to the left. (*b*) Dorsal view of the representation of the barbel in the right side of the dorsal facial lobe.

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