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RESEARCH ARTICLE

Visualizing a set of olfactory sensory neurons responding to a bile salt

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SUMMARY

In the present study, we exposed the olfactory epithelia of crucian carp, *Carassius carassius*, and brown trout, *Salmo trutta*, to dextran coupled with Alexa dyes together with odorants. Dye uptake was severely reduced after pre-exposure to nocodazole, an inhibitor of microtubule polymerization that impairs endocytosis, supporting the hypothesis that odour-activated olfactory receptor molecules undergo endocytosis. Application of the bile acid taurolithocholate, a potent and specific odorant for fish, resulted in the labelling of a sparse (less than 3%) cell population with the typical morphology of ciliated sensory neurons (CSNs) – long dendrites and cell somata deep in the sensory epithelium. The dye was distributed throughout the sensory neuron, also revealing axons and target glomeruli. Stained axons redistribute at the entrance of the olfactory bulb and terminate in two small target areas, a dorsal and a medial one. These results are consistent with the notion that taurolithocholate is detected specifically by a few ciliated sensory neurons. Application of the olfactory epithelium of brown trout to bile acid stained cells with the appearance of CSNs. Application of an alarm agonist, hypxanthine-3-N-oxide, to crucian carp olfactory organ caused staining of another set of sensory neurons. Furthermore, our results show that odour-induced uptake of a dye can serve to identify the subtype of olfactory sensory neurons responding to a particular odorant, and to pinpoint the target regions of these neurons in the olfactory bulb as a first step to elucidating the neuronal network responding to a particular odour.

Key words: endocytosis, ligand specificity, projection pathways, network, ciliated sensory neurons.

INTRODUCTION

Odour detection in vertebrates is achieved by large families of olfactory receptor genes that are expressed mutually exclusively in a large number of primary sensory neurons (Mombaerts, 2004; Serizawa et al., 2005). Thus, the ligand specificity of olfactory sensory neurons should reflect that of the odorant receptors they express, and may allow investigation of odorant receptor ligand sensitivity *in vivo* as well as *in vitro*. Visualization of the olfactory sensory neurons responding to a particular odorant has been achieved in the peripheral region (Ma and Shepherd, 2000), and in the terminal fields of the olfactory bulb (e.g. Friedrich and Korsching, 1998; Johnson and Leon, 2007; Lancet et al., 1982; Leveteau and MacLeod, 1966). However, it has not been possible to visualize the entire shape of activated olfactory sensory neurons, from their dendritic processes to the axonal terminals in the olfactory bulb, using conventional methods of imaging odour-induced neuronal activation.

Prominent endocytic activities have been described in the sensory neurons, both in the main olfactory organ and in the vomeronasal epithelium by electron microscopy (Bannister and Dodson, 1992; Cuschieri and Bannister, 1975; Darin de Lorenzo, 1970; Graziadei and Bannister, 1967; Kolnberger, 1971). Like other G-protein-coupled receptors (GPCRs) (Ferguson, 2001; Seachrist and Ferguson, 2003; von Zastrow, 2001), odorant receptors appear to be internalized upon activation *via* a clathrin-dependent process, as indicated by studies in channel catfish (Rankin et al., 1999) and mouse (Mashukova et al., 2006). After prolonged odorant exposure, receptors are not targeted to lysosomal degradation but accumulate in recycling endosomes, and adaptation of olfactory receptor neurons to odorants can be abolished by the inhibition of clathrin-mediated endocytosis (Mashukova et al., 2006). It is not clear to what extent the endocytosis observed in these experiments may be ligand specific. In a study on chum salmon, *Oncorhynchus kisutch*, whose olfactory epithelium was exposed to copper solutions, fluorescent styryl dyes (FM dyes) were used as membrane markers, and the authors concluded that the labelling of sensory neurons did not depend on the presence of an odorant (Sandahl et al., 2006).

We found ligand-specific endocytosis in taste receptor cells of brown trout, *Salmo trutta*, and hypothesized that ligand-specific receptor-mediated endocytosis may be a common phenomenon in all types of vertebrate chemosensory neurons (Døving et al., 2009). In the present study we examined this question using mainly crucian carp, which exhibits a distinct organization of the olfactory system (Hamdani and Døving, 2007). In fishes there are three types of sensory neuron; the ciliated and microvillous sensory neurons, and crypt cells. The sensory neurons express different types of odorant receptors and respond to different classes of stimuli (Hansen et al., 2003; Lipschitz and Michel, 2002; Sato and Suzuki, 2001). These classes of sensory neurons send their axons to distinct and segregated regions in the olfactory bulb (Hansen et al., 2003; Koide et al., 2009; Sato et al., 2005). A corresponding segregation of pathways is also observed with the respective projection of neurons in the olfactory tract.

In the present study, we exposed the olfactory organ simultaneously to fluorescent dye and a bile acid (Døving et al., 1980), thought to activate ciliated sensory neurons (Thommesen,

1983) at low concentrations. Animals were studied after different survival times to examine the projection of stained axons towards the olfactory bulb. We employed the odour-induced uptake of a fluorescent dye to visualize the sensory neurons responding to an odorant. We show that after application of bile salt, but not of dye alone, a subset of ciliated sensory neurons becomes stained, whose axons can be followed to their terminals in discrete regions in the olfactory bulb. This staining can be impaired by an inhibitor of microtubule polymerization, consistent with receptor-mediated endocytosis as a mechanism for dye uptake in olfactory sensory neurons. This feature may be useful as a new tool for visualizing all the sensory neurons activated by odours. In particular it will allow identification of the morphological subtype of responsive neurons and concomitantly their target areas in the olfactory bulb. A brief account of parts of the present study has been presented elsewhere (Døving et al., 2008).

MATERIALS AND METHODS

Experimental procedures were approved by the Norwegian Animal Research Authority and were conducted in accordance with the Norwegian Animal Welfare Act of 1974, and the Regulation of Animal Experimentation of 1996. The crucian carp (*Carassius carassius* L.) were collected from a local lake and kept either in the aquaria facilities or in an outdoor pond at the Department of Molecular Biosciences, University of Oslo. The brown trout (*Salmo trutta* L.) were supplied by a local trout hatchery (OFA, Sørkedalen) in Oslo.

To study the ligand specificity of the olfactory sensory neurons, we exposed the olfactory organ to 10 nmol l^{-1} taurolithocholate (TLC) together with different dyes (see below). The crucian carp were anaesthetized by an intraperitoneal injection of 2.5 mg kg^{-1} Alfaxan (Vétoquinol Ltd, Buckingham, UK), and placed in a cradle with tap water irrigating the gills. The olfactory rosettes of anaesthetized fish were exposed to the different solutions for between 5 and 30 min. The flow was ~1 ml min^{-1}. After each application the olfactory cavity was rinsed with distilled water for at least 5 min. Fish were kept alive for up to 2 days in aerated water at room temperature and re-anaesthetized and perfused *via* the heart with 4% buffered paraformaldehyde (phosphate buffer 0.1 mol l^{-1}, pH 7.2). In total, 24 fish were used. After fixation, the olfactory organs with bulb and tracts were dissected free and left in fixative. The specimens were embedded in 12% gelatine solution in 0.1 mol l^{-1} phosphate buffer and sectioned in a vibratome.

In some experiments, we exposed crucian carp for 10 min to 3 kDa dextran conjugated with Alexa 488 (green) together with TLC, and then after a short rinse exposed them for 10 min to 10 kDa dextran conjugated with Alexa 647 (red) together with the alarm agonist hypoxanthine-3-(N)-oxide (H3NO), demonstrating selective staining for two different odorants. The olfactory organs of brown trout were exposed to dextran conjugated with Alexa 488 (green) together with TLC in the same way as crucian carp.

In control experiments the olfactory rosettes of anaesthetized fish were exposed for 10 min to dyes alone. Long range transport of endocytic vesicles is often dependent on molecular motors and microtubules which can be disrupted by nocodazole (Schliwa and Woehlke, 2003). The effect of nocodazole was studied by pre-exposing the olfactory rosette to 35 μmol l^{-1} nocodazole for 20 min at room temperature followed by exposure to 10 nmol l^{-1} TLC and 2 μmol l^{-1} styryl dye FM1-43 for 5 min.

For vibratome sections of the olfactory rosette, the position of the cell body of each stained sensory neurone was categorized by the location of its nucleus within the epithelium. The sensory epithelium was divided into five equal zones (layers) from the surface to the basal lamina, zone 1 being uppermost and zone 5 the region closest to the basal membrane. Thus, the position of each cell soma was assigned to a particular zone.

A confocal microscope (Olympus FluoView 1000, BX61W1, Olympus, Shinjuku, Tokyo, Japan) was used to observe the details of single lamellae or sections in the preparations. Pictures were taken in planes, separated by *z*-axis steps varying between 0.4 and 2 μm. Confocal microscope images were analysed by the software Imaris® (www.bitplane.com). The software ImageJ (NIH, Bethesda, MD, USA) was used to handle information from photographs taken at different depths. Images were imported and stacks added in a *z*-projection with maximum intensity parameter. The following water immersion objectives were used: 10× NA 0.30; 20× NA 0.50; 40× NA 0.80; and 60× NA 0.90. Imaging of lamellae and whole *in situ* preparations was performed using a conventional fluorescence microscope (Olympus, BX50WI) equipped with a digital camera (ProgRes, Jena, Germany). For this microscope a 2× objective NA 0.05 was also used. Counting of stained sensory neurons was carried out with confocal images of olfactory lamellae in areas ranging from 44×10^3 to 360×10^3 μm^2.

Electron microscopy

Small specimens of crucian carp were anaesthetized with Alfaxan as detailed above, decapitated, and the heads placed in a fixative containing 4% formaldehyde, 0.3% picric acid and 0.1% glutaraldehyde in 0.1 mol l^{-1} sodium phosphate pH 7.4 buffer. The skin flaps covering the olfactory rosettes were removed and the upper half of the head was placed on pads for scanning electron microscopy. The preparations were critical point dried, sputtered with a Pt/Ir mixture, and observed in a scanning electron microscope (Jeol JSM 6400, Tokyo, Japan). Ciliated sensory neurons were counted from pictures of surface areas of 450 μm^2.

Chemicals

We used dextrans conjugated with the Alexa dyes 3 kDa anionic Alexa fluor 488 D34682, 10 kDa anionic fixable Alexa fluor 488 D22910 and 10 kDa anionic fixable Alexa fluor 647 D22914, the carbocyanine dye DiO in ethanol solution V-22886, and the styryl dye FM1-43 T35356, all purchased from Molecular Probes (Invitrogen, Carlsbad, CA, USA). TLC T7515 and nocodazole M1404 were purchased from Sigma-Aldrich (St Louis, MO, USA). TLC was prepared as stock solutions of 1 mmol l^{-1} in DMSO and applied at 10 nmol l^{-1}. H3NO was obtained from Menai Organics Limited (Deiniol Road, Bangor, Gwynedd, UK) and prepared by dissolving H3NO in 5 mmol l^{-1} NaCl and 2 mmol l^{-1} Hepes pH 5.3. H3NO was diluted to 0.1 mmol l^{-1} in artificial fresh water pH 7.4. The final H3NO concentration was 10 nmol l^{-1}. All solutions were stored at –20°C.

RESULTS

Outline of the sensory epithelium

The olfactory rosette of crucian carp consists of a series of lamellae (4–8) extending pinnately from a central raphe. In scanning electron micrographs (SEM), the surface of a lamella is divided into an outer region with motile kinocilia (K) (Fig. 1A) and an inner region with sensory neurons. In this inner region we find small islets where the kinocilia dominate (S). The regions with and without kinocilia are called ‘non-sensory’ and ‘sensory’ epithelium, respectively.

Exposing the olfactory organ of crucian carp to TLC (10 nmol l^{-1}) together with one of the dextrans for 10 min resulted in staining of a number of sensory neurons (Fig. 1B). The islets

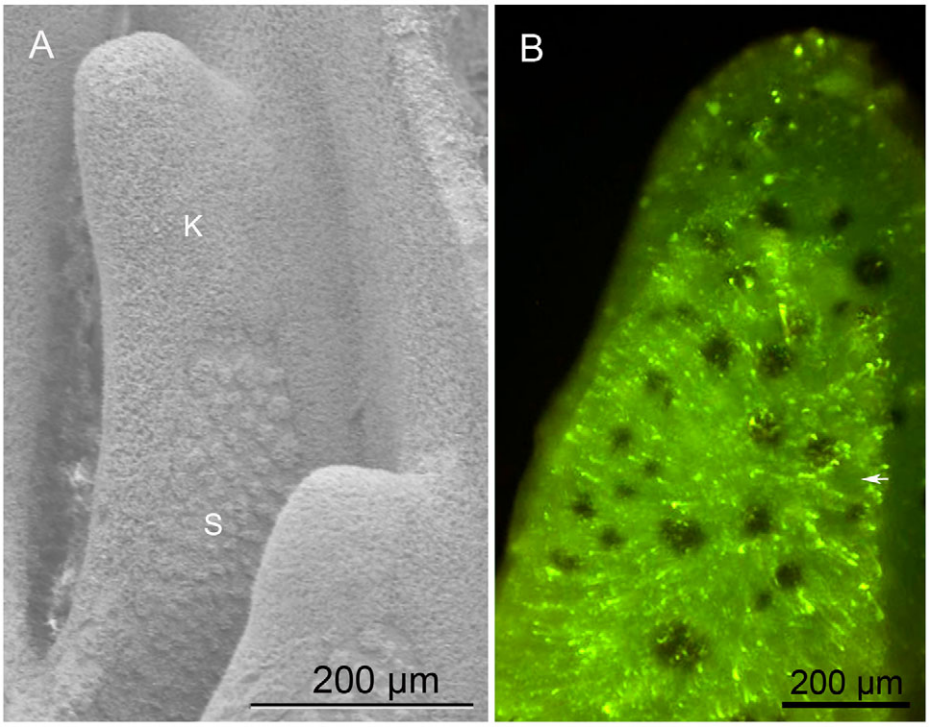

Fig. 1. Lamellae of the olfactory rosette. (A) Scanning electron micrograph (SEM) showing lamellae of the olfactory rosette in crucian carp. The outer rim of a lamella is made up of a region with kinocilia (K); the inner region has a high density of sensory neurons and there are islets with kinocilia (S). (B) Fluorescence micrograph of a lamella from a rosette exposed *in vivo* to 10 nmol l^{-1} taurolithocholate (TLC) and 10 µmol l^{-1} 10 kDa dextran conjugated with Alexa 488 for 10 min. Arrow indicates an area without stained cells that might be islets with kinocilia.

are presumably regions with kinocilia, and can be seen as dark areas in Fig. 1B. The stained neurons were mostly found in the 'sensory' region of the lamellae, but some sensory neurons were also seen in the 'non-sensory' regions. The distribution of stained neurons did not show signs of clustering and all lamellae had stained sensory neurons.

Endocytosis

Application of the potent odorant TLC at 10 nmol l^{-1}, together with 10 µmol l^{-1} 10 kDa dextran conjugated with Alexa 488, caused prominent staining of a restricted number of sensory neurons (Fig. 2A). Application of the dextran dye without TLC did not lead to staining of any of the cells in the epithelium (Fig. 2B). Similar results were obtained using the carbocyanide dye DiO. The use of the styryl dye FM1-43 caused staining of a few cells when applied without TLC. Application of TLC (10 nmol l^{-1}) together with FM1-43 (2 µmol l^{-1}) caused staining as shown in Fig. 2C. Because we suspected that the staining of sensory neurons was induced by endocytosis, we pre-exposed the olfactory rosette to nocodazole, which is known to disrupt microtubules. Pre-exposure of the olfactory organ to nocodazole (35 µmol l^{-1}) for 20 min followed by exposure to TLC (10 nmol l^{-1}), together with FM1-43 (2 µmol l^{-1}), resulted in a dramatic reduction in the number of sensory neurons stained (Fig. 2D), demonstrating that vesicle transport is inhibited.

The great majority of sensory neurons had long dendrites and the cell bodies were deep in the sensory epithelium. The sensory neurons with long dendrites are considered to be ciliated (Hamdani and Døving, 2002; Morita and Finger, 1998) and, in some preparations, labelled cilia extended from the apical pole (Fig. 3).

An additional feature made us believe that entry of the dye occurred *via* endocytosis; the staining was frequently seen as bright spots within the cell. However, there were occasions where the staining was homogeneously distributed within the cell. One case was in the cilia (Fig. 3) and the other was in the axons in the olfactory nerve (Fig. 5A).

Density of sensory neurons

To estimate the proportion of ciliated sensory neurons (CSNs) that had been stained by application of TLC, we compared the densities of CSNs from SEM and compared these densities with those obtained from confocal images. In SEM of the olfactory lamellae,

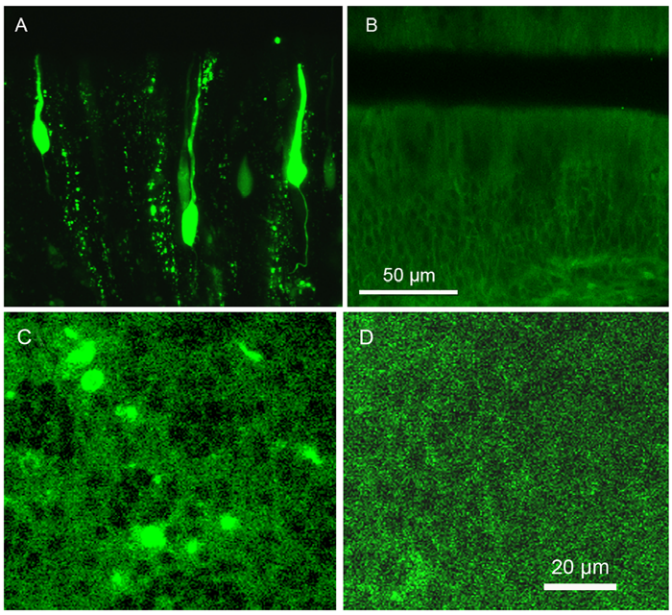

Fig. 2. Control experiments. Images from sensory epithelia of crucian carp exposed for 10 min to 10 kDa dextran conjugated with Alexa 488 with (A) and without TLC (B). (A) The image illustrates three stained neurons with long dendrites. The image is a *z*-projection of a stack of 70 pictures, *z*-step 0.4 µm. (B) Image of a *z*-projection of 10 pictures, *z*-step 0.4 µm. Exposure of the olfactory rosette to 10 nmol l^{-1} TLC and the styryl dye 2 µmol l^{-1} FM1-43 caused staining of sensory neurons (C). When the olfactory rosette was exposed to nocodazole before application of 10 nmol l^{-1} TLC and 2 µmol l^{-1} FM1-43 there were few stained cells (D).

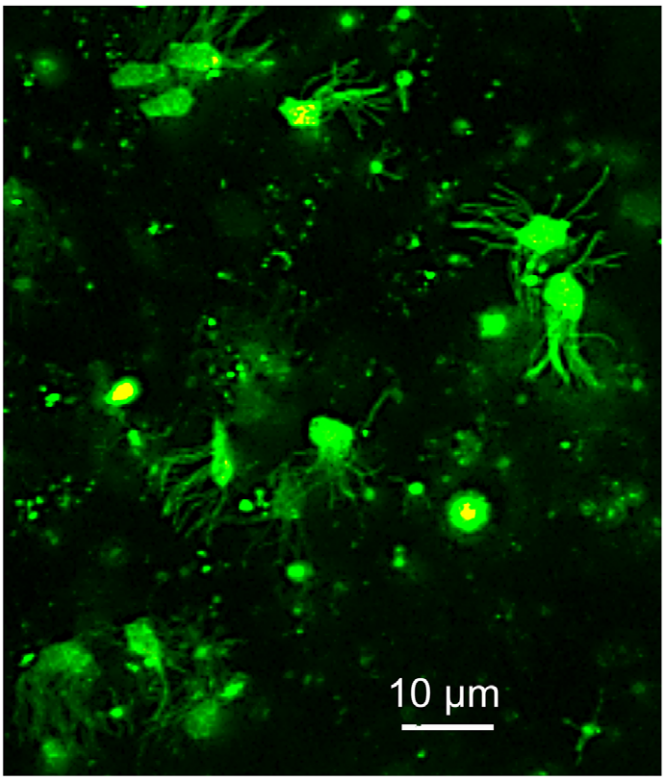

Fig. 3. Confocal image of the apical part of the sensory neurons. *z*-projection of the upper part of the sensory epithelium from an olfactory rosette of crucian carp exposed for 10 min to TLC together with 10 kDa dextran conjugated with Alexa 488, demonstrating staining of the sensory cilia. The image is a *z*-projection of 16 pictures, *z*-step 0.4 µm.

the ciliated sensory neurons are easily recognized. Based on counts of ciliated sensory neurons in SEM micrographs in the 'sensory' regions, the densities were estimated to be $163.8\times10^3\pm13.5\times10^3$ cells mm^{-2} (N=4). In the preparations exposed to TLC and dye, the densities of stained sensory neurones were estimated to be around $4.3\times10^3\pm3.5\times10^3$ cells mm^{-2} (N=9). These numbers indicate that TLC stained about 2.6% of the ciliated sensory neurons.

In olfactory rosettes that had been exposed to exposed to TLC and 10 kDa dextran conjugated with Alexa 488, we estimated the position of the cell soma of each stained sensory neuron. In vibratome sections, the position of the cell body of each stained sensory neuron was categorized by the depth of its nucleus within the epithelium. Of 237 neurons stained, the percentages of cell somas found in the different zones were: 0.4% in zone 1, 3.4% in zone 2, 18.6% in zone 3, 67.1% in zone 4 and 10.5% in zone 5.

Axons

The axons from the sensory neurons leave the olfactory epithelium (Fig. 4) from single sensory neurons. These fibres could be followed in the lamellae, the olfactory nerve and the bulb. We investigated the course of these stained fibres in crucian carp that had survived for up to 48 h. In the olfactory nerve the axons run in parallel (Fig. 5A). We believe that the axons seen are single entities, as they have the same appearance near the sensory neuron as in the olfactory nerve. Note that the dye is distributed in spots, but also as a homogeneous stain along an axon (Fig. 5A). It should be recalled that the axons of the olfactory sensory neurons are unmyelinated fibres (Døving and Gemne, 1969; Gasser, 1956) and that there are several tens of axons packed within one mesaxon in the olfactory nerve. Should all sensory neurons have taken up the dye and all the axons become stained, it would have been impossible to distinguish individual fibres. We believe that this observation also indicates that a subset of sensory neurons was stained.

The axons forming the olfactory nerve come from all parts of the olfactory rosette and as they approach the rostral part of the olfactory bulb they are redistributed. In a transverse section of this part of the olfactory bulb the axons stained using TLC run parallel to the plane of section and gather in one region of the neuroplexus (Fig. 5B).

More posteriorly in the bulb, the axons run as small bundles until they enter the deeper layers of the bulb (Fig. 6A and Fig. 7). The axons terminate in two regions of the olfactory bulb that we consider to be glomerular regions. To study how the axons terminate in the olfactory bulb, we exposed both olfactory epithelia of the fish to the odorant TLC and dye for 10 min. The fish had been kept alive for 8 h post-dye application. We found that the stained axons enter the dorsal part of the olfactory bulb symmetrical on the two sides (Fig. 6B). In addition, a branch of axons terminated in the medial region of the bulb (Fig. 7). In summary, these findings confirm the specificity of the dye uptake.

Specificity

It might be argued that the staining we observe is due to particular features of TLC or the species that we have used. To address such arguments, we applied another odorant, the alarm agonist H3NO (Argentini, 1976), known to induce alarm reaction in several species (Brown et al., 2001; Parra et al., 2009; Pfeiffer et al., 1985). In experiments where we exposed the olfactory organ of crucian

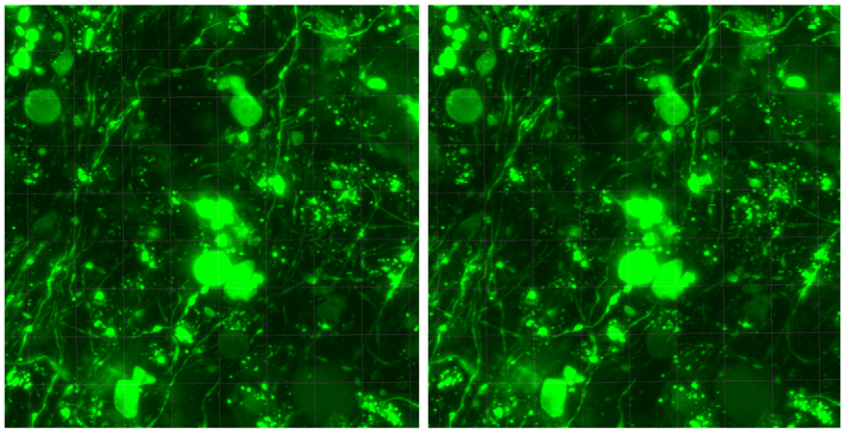

Fig. 4. Stereograph of axons leaving the sensory neurons. The pair of images was made with Imaris software from confocal micrographs of the lower part the sensory epithelium and below the basal lamina. The olfactory rosette of crucian carp was exposed for 10 min to TLC together with 10 kDa dextran conjugated with Alexa 488. Note the axons running from the single sensory neurons.

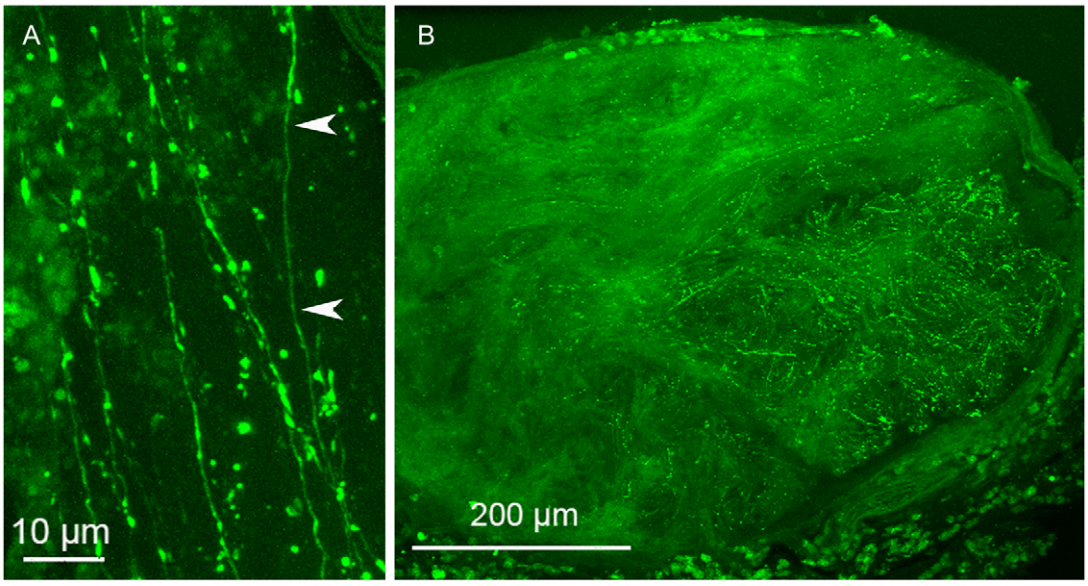

Fig. 5. Axons of sensory neurons in the olfactory nerve and the olfactory bulb. (A) Confocal images taken parallel with the axis of the olfactory nerve from a preparation where the olfactory rosette in crucian carp was exposed for 10 min to TLC together with 10 kDa dextran conjugated with Alexa 488. The image is a *z*-projection of 25 confocal pictures, *z*-step 0.4 µm. Note that in the axons there are both spots with high fluorescence and regions with homogeneous staining, e.g. between the two arrowheads. (B) At the interface between the olfactory nerve and bulb the fibres are seen to run parallel to this transverse section to aggregate towards their terminations in the glomerular region. Note that the majority of axons are in the dorso-medial region of the bulb, at the right of the picture.

carp first to TLC together with 3 kDa dextran conjugated with Alexa 488 (green) and then to the alarm agonist H3NO together with 10 kDa dextran conjugated with Alexa 647 (red), we found staining in two different sets of olfactory sensory neurons (Fig. 8A).

In other experiments, we exposed the olfactory organ of brown trout to 3 kDa dextran conjugated with Alexa 488 together with TLC. In this species too, the procedure caused staining of ciliated sensory neurons with long dendrites (Fig. 8B). The projections in the olfactory bulb were not studied.

DISCUSSION

The present study describes an odour-specific uptake of dye in the ciliated sensory neurons of the olfactory epithelium in crucian carp. These findings demonstrate that it is possible to visualize the network of neurons responding to an odorant. The ability to stain the sensory neurons responding to particular odorants will enable a deeper understanding of the olfactory system.

Selectivity

Several findings in our study indicate an odour-specific dye uptake. One set of observations was that the majority of stained sensory neurons (67%) had their cell body in zone (layer) 4, that they had cilia, and that less than 3% of the CSNs were stained. Further, the olfactory nerve consists of a large number of axons; and there are several tens of axons packed within a single mesaxon (Døving and Gemne, 1969; Gasser, 1956), but in confocal micrographs we observed only single axons extending from sensory neurons. Our studies reveal that the axons of the stained cells terminate in small regions of the olfactory bulb. This finding is in accordance with previous data showing that the sensory neurons expressing a particular odorant receptor terminate in specific glomeruli. This feature has been shown to be the case both in mammals (Ishii et al., 2001; Ressler et al., 1994; Vassar et al., 1994) and fish (Hansen et al., 2003; Sato et al., 2005; Sato et al., 2007). A functional projection has also been found using voltage-sensitive dyes

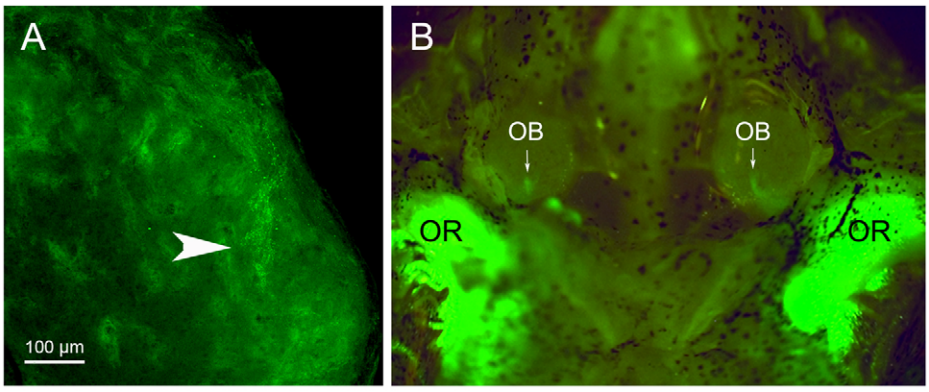

Fig. 6. Axons terminating in the olfactory bulb. (A) In a transverse section of the olfactory bulb in crucian carp, axons are seen to leave the peripheral layer of the bulb to enter the glomerular region (arrowhead). (B) Dorsal view of the head of a crucian carp exposing the olfactory rosettes (OR) and the olfactory bulbs (OB). The terminal fields of stained axons in the bulb are indicated by arrows. In order to view the terminal fields, the olfactory rosettes are overexposed. The olfactory bulbs are about 1.2 mm in diameter.

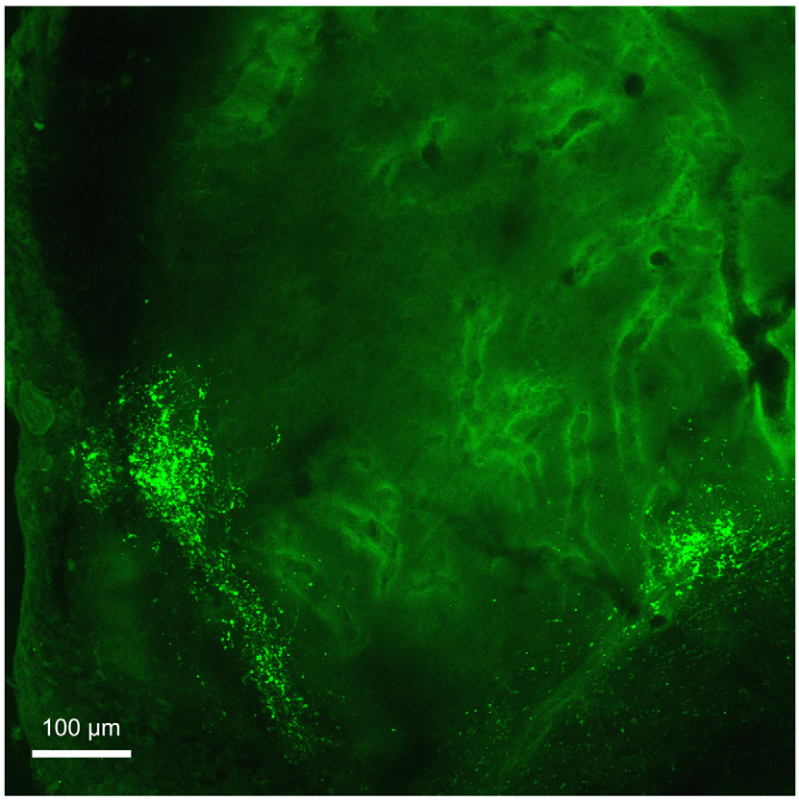

Fig. 7. Terminal fields of axons. Oblique section of the olfactory bulb in crucian carp. Two terminal fields of the axons of sensory neurons responding to the bile salt TLC. The region to the left is the dorsal site and the region to the right is the medial site. The image is a *z*-projection of 80 confocal pictures, *z*-step 0.4 µm.

(Friedrich and Korsching, 1997; Friedrich and Korsching, 1998). The selective staining was not due to particular features of the odorant used, as an alarm agonist gave staining in another set of sensory neurons. Further, the bile salt TLC also induced staining of sensory neurons with long dendrites in brown trout. Thus, similar neural elements were identified in fish species from two different orders.

Endocytosis

In mice, it has been shown that odorant receptors are internalized *via* clathrin-dependent endocytosis (Mashukova et al., 2006). The authors of that study also showed that prolonged odorant exposure led to the accumulation of receptors in recycling endosomes, and not to lysosomal degradation. The staining of axons indicates a prominent transcytosis in the olfactory sensory neurons (Hocini et al., 2001; Miller et al., 1994). It is tempting to associate such a transcytosis with the essential role of odorant receptors in targeting axonal projection to precise sites in the olfactory bulb (Miyamichi et al., 2005; Tsuboi et al., 2006; Wang et al., 1998). The participation of odorant receptors in targeting requires transport of these receptors to the axon terminals, and ligand-specific endocytosis may play a part in such processes.

It is remarkable that freshly exposed preparations (less than 1 h) in this study showed both spots of staining within the cytoplasm of the sensory neuron and also regions where the staining was homogeneous in the cytoplasm of the cell proper and in the axons. If the dyes are initially internalized as endosomes, this implies that the dye has subsequently been released from the endosomes. It has been suggested that FMI-43 injected into mice enters sensory neurons through non-selective ion channels (Meyers et al., 2003). This would also give a homogeneous staining, but the idea that the 10 kDa dextran could pass through ion channels is questionable.

Transport

The bright spots of stain and homogeneous staining of axons of sensory neurons reveal different stain-transporting properties. As mentioned above, the homogeneous staining suggests that the dye has been released from endosomes, and diffuses into the cytosol to

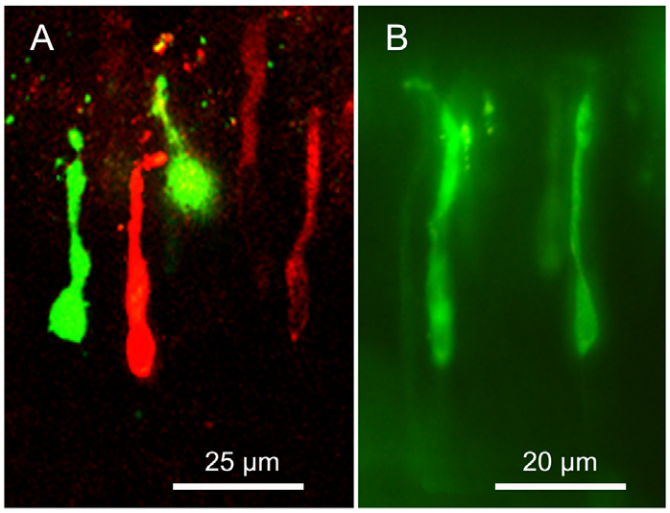

Fig. 8. (A) *z*-projection of eight images, *z*-step 0.4 µm, of the sensory epithelium from an olfactory rosette of a crucian carp exposed for 10 min to TLC together with 10 kDa dextran conjugated with Alexa 488 (green) and then exposed for 10 min to the alarm agonist hypoxanthine-3-(N)-oxide (H3NO) together with 10 kDa dextran conjugated with Alexa 647 (red), demonstrating selective staining for two different odorants. (B) Image of the sensory epithelium of a brown trout, exposed for 10 min to TLC together with 10 kDa dextran conjugated with Alexa 488 (green). In this species, TLC induces staining in sensory neurons with long dendrites.

be transported as dextran molecular entities. The odorant receptors seem further to participate in the targeting of the axons of the sensory neurons (Miyamichi et al., 2005; Nakatani et al., 2003).

The choice of dye in the present study was prompted by the many uses of dextran in studies of axonal transport (Boulton et al., 1992; Fritzsch, 1993; Glover et al., 1986; Raju and Smith, 2006). Stain was seen in patches, indicating endosomes. However, as the stain was also seen as homogeneous stretches in the axons, the dextrans must have been transported as molecular entities.

Ligand

TLC was selected because bile salts have been shown to be potent odorants for salmonids (Døving et al., 1980), crucian carp (Lastein et al., 2008) and catfish (Rolen and Caprio, 2007), and are considered to activate ciliated sensory neurons (Thommesen, 1983). Bile salts have been suggested to play a role in the homing behaviour of salmonids (Døving et al., 1980), and bile salts have been shown to act as migratory pheromones for the sea lamprey (Bjerselius et al., 2000; Fine and Sorensen, 2005; Fine et al., 2004; Sorensen et al., 2005). The results of the present study confirm the previous proposal that bile salts activate the ciliated sensory neurons (Thommesen, 1983).

CONCLUSION

Odour-specific endocytosis is a fundamental and vital feature of olfactory sensory neuron function. The experimental exploitation of this feature will have implications for our understanding of the olfactory system, and its application provides a new tool for visualizing sensory neurons responding to an odorant. The ligand-specific induction of endocytosis can be used to reveal the molecular identity of odorant receptors that respond to particular odorants. By using dyes that are transported across synapses in conjunction with a specific odorant, it will be possible to uncover the nervous pathways for a specific behaviour that is released by such an odorant.

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