Glomerular Territories in the Olfactory Bulb from the Larval Stage of the Sea Lamprey Petromyzon marinus

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ABSTRACT

The goal of this study was to investigate the spatial organization of olfactory glomeruli and of substances relevant to olfactory sensory neuron activity in the developing agnathan, the sea lamprey Petromyzon marinus. A 45-kD protein immunoreactive to G\textsubscript{olf}, a cAMP-dependent olfactory G protein, was present in the ciliary fraction of sea lamprey olfactory epithelium and in olfactory sensory neurons of larval and adult sea lampreys. This result implies that G\textsubscript{olf} expression was present during early vertebrate evolution or evolved in parallel in gnathostome and agnathostome vertebrates. Serial sectioning of the olfactory bulb revealed a consistent pattern of olfactory glomeruli stained by GS1B\textsubscript{4} lectin and by anterograde labeling with fluorescent dextran. These glomerular territories included the dorsal cluster, dorsal ring, anterior plexus, lateral chain, medial glomeruli, ventral ring, and ventral cluster. The dorsal, anterior, lateral, and ventral glomeruli contained olfactory sensory axon terminals that were G\textsubscript{olf}-immunoreactive. However, a specific subset, the medial glomeruli, did not display this immunoreactivity. Olfactory glomeruli in the dorsal hemisphere of the olfactory bulb, the dorsal cluster, dorsal ring, anterior plexus, lateral chain, and medial glomeruli, were seen adjacent to 5HT-immunoreactive fibers. However, glomeruli in the ventral hemisphere, the ventral ring, and ventral cluster did not display this association. The presence of specific glomerular territories and discrete glomerular subsets with substances relevant to olfactory sensory neuron activity suggest a spatial organization of information flow in the lamprey olfactory pathway. J. Comp. Neurol. 465:27–37, 2003.

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In all vertebrates, from agnathans to primates, axons of olfactory sensory neurons (OSNs) project from soma in the olfactory epithelium to olfactory bulb glomeruli (Iwahori et al., 1987; Halasz and Greer, 1993; Klenoff and Greer, 1998; Lin and Ngai, 1999). Although functional differences in OSNs that terminate in specific glomerular territories have been observed, glomerular organizing principles are not fully understood (Hildebrand and Shepherd, 1997; Xu et al., 2000). The arrangement of OSN axon terminals into glomerular units may be affected by transduction mechanisms fundamental to OSN function and by modulatory influences from nonolfactory neuronal fibers adjacent to OSN axons. The cAMP-dependent olfactory GTP-binding protein linked to olfactory receptors (Jones and Reed, 1989), G_{olf}, is a constituent of olfactory sensory transduction and a requisite for olfactory responses in mammals (Belluscio et al., 1998). Because OSN subpopulations in amphibia (Mezlier et al., 2001) and teleosts (Hansen et al., 2001) express G_{olf}, this G_{olf} subunit may have appeared early in vertebrate evolution. Alternate G-proteins are found in venuromesural sensory neurons (Jia and Halpern, 1996) and in OSN subpopulations of amphibia (Mezlier et al., 2001) and teleosts (Hansen et al., 2001). If G_{olf} is a vital component for vertebrate OSN function, its localization in the olfactory organ of the sea lamprey, an ancestral jawless fish, is expected. If transduction by alternate G-proteins is also a fundamental character or if it evolved in parallel (Eisthen, 2002), clearly defined subpopulations of OSNs that do not express G_{olf} should be present in the lamprey.

Previous studies have provided evidence that OSN terminals are distributed according to functional parameters. In mammals, axons of OSNs expressing putative odorant receptors within an epithelial zone converge to spatially conserved glomeruli (Mombaerts, 1996). In addition, OSNs containing elements of a cGMP signal transduction pathway project to specific glomeruli (Juilfs et al., 1997) and identified odorants stimulate synaptic activity in specific glomeruli (e.g., Wachowiak and Cohen, 2001). In zebrafish (Teleostei, Cypriniformes), olfactory glomeruli are arranged in a stereotyped configuration (Baier and Korsching, 1994) and glomerular responsiveness to odor stimulation follows spatial patterning (Friedrich and Korsching, 1998). In catfish (Teleostei, Siluriformes), two OSN subpopulations project axons to specific regions of the olfactory bulb (Morita and Finger, 1998), and in the lamprey, Lampetra fluviatilis, calretinin-immunoreactive OSNs extend to particular glomerular locations (Pombal et al., 2002).

In view of these examples of spatially and functionally distinct glomerular arrangements, we propose that subpopulations of OSNs expressing the G-protein, G_{olf}, extend axons into spatially distinct glomeruli in the lamprey. Earlier reports have shown a ring-like arrangement of olfactory glomeruli in Lampetra fluviatilis and L. planeri (Schober, 1964), Ichthyomyzon unicuspinus (Northcutt and Puzdrowski, 1988), Petromyzon marinus (Tobet et al., 1996), and Lampetra japonica (Iwahori et al., 1997). However, details of the arrangement of the glomerular territories in lampreys are lacking. The olfactory receptor family is relatively small in lampreys (Berghard and Dryer, 1998; Freitag et al., 1999) and, not surprisingly, few odorants stimulate olfactory activity (Li and Sorensen, 1995). These findings suggest that the larval lamprey has considerably fewer olfactory glomeruli than the 1,800–2,400 glomeruli located in the mammalian olfactory bulb (Royet et al., 1988; Meisami and Sendera, 1993) and that mapping of glomerular territories is feasible in serially sectioned preparations of the lamprey olfactory bulb.

In sea lamprey, nonolfactory serotonergic nerve fibers are present along the primary olfactory pathway and enter the olfactory bulb (Zielinski et al., 2000). These fibers may be associated with particular olfactory sensory input and may innervate specific glomerular regions. Therefore, the goal of this study was to probe the olfactory bulb of the developing lamprey for spatially conserved glomerular territories and for the distribution of two substances that may be relevant to OSN activity: the protein G_{olf} and the biogenic amine neurotransmitter serotonin.

In this study we observed six glomerular territories, G_{olf}, immunoreactivity at specific glomerular sites, and distinct glomerular subsets with serotonergic nonolfactory fibers. These results imply differences in transduction mechanisms for some OSNs and modulation of specific OSNs by biogenic amines.

**MATERIALS AND METHODS**

**Experimental animals**

Year two and three class larval sea lampreys were obtained from naturally occurring populations collected from creeks in Michigan, then housed at the Lake Huron Biological Station, Millersburg, Michigan, or from Oshawa Creek and Bronte Creek, Ontario. All larval lampreys were maintained at 10°C at the Department of Biological Sciences, University of Windsor. Approximately 90 larvae were used for this study (total length 80–130 mm, weight 0.6–2.7 g). Adult sea lampreys were trapped or collected by hand from tributaries to Lakes Huron and Michigan, transported to the U.S. Geological Survey Lake Huron Biological Station, Millersburg, Michigan. These adults were held in flow-through tanks (1,000 L) with Lake Huron water (7–20°C) before being euthanized for collection of olfactory organs. Prior to experimentation, all lampreys were deeply anesthetized with tricaine methane sulfonate (MS-222) and killed by decapitation. All experimental protocols reported in this study were in compliance with guidelines established by the Canadian Council of Animal Care.

**Western immunoblot**

The cilia were dissociated from OSNs by calcium shock (Schandar et al., 1998). In brief, sea lamprey olfactory
epithelia were dissected out and agitated in a high calcium buffer (10 mM CaCl₂, 20 mM ACES, 0.3 M sucrose, 10 μg/ml leupeptin, 76.8 nM apro tin, 0.7 μM pepstatin, 0.83 mM benzamide, 0.23 mM PMSF, 1 mM iodoacetamide) in an end-over-shaker at 4°C for 20 min. The solution was spun for 15 min at 6,000 g and the supernatant was collected. The pellet was resuspended in the same buffer and spun down again for collection of the supernatant. The combined supernatant was spun for 15 min at 18,000 g to isolate the cilia. The ciliary pellet was washed with TME buffer (10 mM Tris, 3 mM MgCl₂, 2 mM EGTA, pH 8.2) and resuspended. Aliquots were stored at −80°C until use. The deciliated olfactory mucosa was prepared from the pellet of the first centrifugation according to DellaCorte et al. (1996). The deciliated tissue was homogenized using a mortar and pestle and centrifuged at 30,000 g for 90 min at 0°C. The supernatant was removed and stored at −80°C. The protein concentration was determined by DCA protein analysis kit (Pierce Biotechnology, Rockford, IL). Ten and 20 μg protein of cilia and deciliated mucosa were loaded onto a 10% SDS PAGE gel and 5% stacking gel which were subjected to 150 V for 1 hour in a standard running buffer. The protein bands in the gel were transferred to a PVDF membrane. The membrane was then blocked with a blocking solution containing 5% nonfat dry milk in buffer, incubated with the primary antibody (anti-G₁ subunit 1:200 or 1:500) in the blocking solution, washed three times with buffer, and incubated with HRP-conjugated secondary antibody (1:5,000) in the blocking solution. Finally, the membrane was washed three times and incubated in chemiluminescence substrate for 10–15 minutes and film was exposed and developed.

**Tissue fixation**

The larval heads were immersed in Zamboni’s fixative for 4–20 hours (2% paraformaldehyde, 1.5% picric acid, 0.1 M phosphate buffer, pH 7.4) at 4°C, then cryoprotected by passage through a sucrose gradient (10–20–30% in PB). Horizontal sections (20–30 μm) were cut on a cryostat (Microm). Sections were air-dried for at least 1 hour, postfixed in acetone at −20°C for 10 minutes, and rehydrated with 0.1 M phosphate buffered saline (PBS) (pH 7.4) for 10 minutes at room temperature.

**GS1B₁ lectin histochemistry**

The staining of larval sea lamprey olfactory glomeruli by *Griffonia simplicifolia*-1 (GS-1 isoelectric B₁) was previously described (Tobet et al., 1996; Zielinski et al., 2000). Slides were incubated with biotin-conjugated lectin, *G. simplicifolia*-1 (GS-1 isoelectric B₁, Vector, Burlingame, CA;
10 μg/ml in 0.1 M PBS, pH 7.5) for 3 hours. These were rinsed in PBS, then incubated in DCS avidin-fluorescein (1:100 in 0.1 M bicarbonate buffer, pH 8.5) for 1 hour, washed, then mounted with Vectashield.

Immunocytochemistry

Slides with tissue sections were incubated in diluted normal horse serum for 20 minutes, then in primary antiserum raised in rabbits against GS1B (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA) or serotonin (5-HT, 1:3,000; DiaSorin, Stillwater MN) in 0.1 M PBS, pH 7.4, containing 0.1% Triton X-100 overnight at 4°C. The sections were rinsed in PBS, incubated in Alexa 568 goat antirabbit IgG (Molecular Probes, Eugene, OR; 1:100 in PBS, pH 7.4) for 1 hour and rinsed in PBS. Negative controls, with primary antibody omitted from the staining procedure, were included with each immunocytochemical preparation. Specificity of the 5HT antibody was tested by omitting this primary antibody from the staining protocol and by a preadsorption control experiment. Preadsorption control preparations for 5-HT were prepared with diluted antiserum that had been preadsorbed with 10 mM or 100 mM 5HT (Sigma, St. Louis, MO) for 24 hours at 4°C.

Application of calcium green dextran

The glomerular units containing OSN projections in the olfactory bulb were anterogradely labeled following the application of calcium green dextran into the nasal cavity. Prior to experimentation all lampreys were individually anesthetized by immersion in a 0.05% solution of MS222. After the animal was sedated it was wrapped in wet tissue paper. A solution of 2.5% calcium green 1-dextran (potassium salt 3,000 MW anionic, Molecular Probes), 0.2% TritonX-100, and 1 mM NaCl, 0.1 M Na bicarbonate in water was injected into the nasal cavities using a Hamilton syringe with a 23 gauge needle. Following this treatment the lamprey recovered and another injection was applied the following day. On the fifth day following this injection the lamprey was immersed in a solution of MS222, deeply anesthetized, and killed by decapitation.

The larval heads were immersed into 4% paraformaldehyde fixative overnight at 4°C, then cryoprotected by passage through a sucrose gradient (10–20–30% in PB). Cryosections (20 μm) were cut in horizontal planes on a cryostat (Microm). Coverslips were mounted with Vectashield (Vector). The sections were viewed by fluorescence microscopy or confocal microscopy (BioRad 1024, Hercules, CA).

Morphometry

Larval olfactory bulbs prepared for GS1B4 lectin histochemistry, fluorescent dextran, Gαs, and 5HT were serially sectioned and stained (n = 25). These sections were viewed on an Axioskop (Zeiss) and prepared as a 3D movie using Empix Eclipse software (Mississauga, Canada). This computer program was used to measure the glomerular diameters and the total glomerular volume. The number of olfactory glomeruli olfactory bulbs was estimated by applying the dissector technique (Gundersen et al., 1988) to the 3D reconstruction of the serially sectioned olfactory bulbs.

Production of photomicrographs

The confocal images were acquired in BioRad PIC format and converted to TIFF format with Confocal Assistant. The fluorescence microscopy images were acquired using Empix Eclipse software and saved in TIFF format. The photomicroscope images were assembled into figures and labeled with Adobe PhotoShop (Mountain View, CA).

RESULTS

Western blotting of the ciliary fraction from adult lamprey olfactory epithelium demonstrated the specificity of the Gαs antibody. The molecular weight of Gαs in the cilia of lamprey OSNs was 45 kDa, with a slightly higher molecular weight component likely from the phosphorylated form of this G-protein (Fig. 1). The strong Gαs immunoreactive (IR) band in the ciliary fraction is supported through immunolocalization. Ciliary localization was seen in double-labeled preparations with acetylated tubulin and Gαs antibodies (Fig. 2A,B). Acetylated tubulin immunoreactivity indicated the presence of cilia (Fig. 2A). In larvae, the boundary between the olfactory epithelium and non-Gαs-expressing nasal epithelium was sharp and clear (Fig. 2B). High-power views confirmed strong Gαs-IR in cilia of OSNs (Fig. 2C). Gαs expression in cilia, dendrites, cell bodies, and axons persisted after metamorphosis (Fig. 2D), when OSNs were considerably larger than during larval development (Vandenbossche et al., 1995, 1997). OSN subcellular components located in the olfactory epithelium, dendrites, soma, and axons contained Gαs-IR (Fig. 2D). The presence of Gαs immunoreactivity in the OSN axons led us to examine spatial distribution of Gαs-IR axons in the olfactory bulb.

Glomerular territories

The detailed glomerular organization in the lamprey olfactory bulb has not been documented. Therefore, we mapped the olfactory bulbar neuropil innervated by OSNs before charting Gαs-IR glomeruli. Examination of the olfactory bulb from serial sections in the horizontal plane, stained with GS1B4 lectin (Fig. 3), and by anterograde labeling with fluorescent dextran, revealed a consistent pattern of glomerular organization. All glomeruli that were identified were labeled by both techniques. The spa-
tial distribution of the glomerular groupings was consistent in the larvae examined (80–130 mm long). The thickness of the olfactory bulb was ~500 μm in the smaller lampreys. Spatial analysis is presented in a series of evenly spaced sections through the olfactory bulb of larvae with a length of 12–13 cm, where the thickness of the olfactory bulb was 725–800 μm. The olfactory nerve entered the olfactory bulb at a depth of 300–425 μm. The diameter of olfactory glomeruli ranged from 45–90 μm. Tallies of glomerular modules in serially sectioned larval olfactory bulbs indicated that the total number of glomeruli ranged from 41–65 (n = 10). In addition to forming olfactory glomeruli, OSN fibers were seen entering the olfactory bulb from the olfactory nerve and proceeding medially beyond the olfactory bulb to the ventral diencephalon, as previously reported (Tobet et al., 1996).

We consistently observed the following glomerular territories in larval sea lamprey.

**Dorsal cluster, 40–100 μm.** A cluster of 6–10 olfactory glomeruli was apparent close to the dorsal edge of the olfactory bulb (Fig. 3A).

**Dorsal ring, 100–200 μm.** Approximately 7–10 closely spaced glomerular modules were arranged in a circular pattern along the medial, anterior, and lateral edges and the olfactory nerve layer was prominent at the anterior and lateral edge (Fig. 3B).

**Anterior plexus, 225–475 μm.** The anterior plexus consisted of a cohesive mesh of OSN axon terminals (Fig. 3C–E). Intense GS1B₄ labeling at the medial edge indicated the location of olfactory nerve fibers entering the olfactory bulb.

**Lateral chain, 225–475 μm.** A space about 50 μm wide separated the anterior plexus from the lateral chain, a relatively narrow group of glomerular modules extending along the lateral edge of the olfactory bulb (Fig. 3C–E). The anterior plexus and lateral chain were previously observed in the zebrafish olfactory bulb (Baier and Korshing, 1994).

**Medial glomeruli, 350–575 μm.** At bulb depths of 350–425 μm, medial glomeruli were positioned posterior to the junction of the olfactory nerve and olfactory bulb (Fig. 3D,E). At 350 μm, a small anterior-radial glomerulus was positioned posterior to the medial edge of the anterior plexus; a second medial glomerulus, the posterior-radial glomerulus, was positioned radially (Fig. 3D), and a third glomerulus faced the anterior commissure (Fig. 3D,E). Medial glomeruli were located ventral to the olfactory nerve (450–600 μm); a posterior glomerulus and a glomerulus adjacent to the anterior commissure (Fig. 3F,G).

**Ventral ring, 500–675 μm.** A circular arrangement of 10–14 glomerular units were present in sections in the olfactory bulb taken ventral to the olfactory nerve at depths of 500–600 μm (Fig. 3F–H). At 500 μm, GS1B₄-positive fibers were seen extending from anterior glomeruli of this ring into the granular layer.

**Ventral cluster, 700–750 μm.** The bottom portion of the olfactory bulb contained a cluster of 7–10 olfactory glomeruli (Fig. 3I).

**GloMerular Gₛₐᵣ-immunolabelling.** Glomerular territories in the dorsal, anterior, lateral, and ventral regions of the olfactory bulb contained Gₛₐᵣ-IR. However, the medial glomeruli were not Gₛₐᵣ-IR (Fig. 4). The Gₛₐᵣ-immunolabeling was also intense in the olfactory nerve and the olfactory nerve layer of the olfactory bulb.

**Dorsal cluster and dorsal ring.** The glomerular units in this dorsal region of the olfactory bulb contained Gₛₐᵣ-immunoreactivity (Fig. 4A).

**Anterior plexus and lateral chain.** These glomeruli contained Gₛₐᵣ-immunoreactivity (Fig. 4B).

**Medial glomeruli.** Gₛₐᵣ-immunoreactivity was absent from the region occupied by medial glomeruli. In preparations double-labeled with Gₛₐᵣ immunocytochemistry and anterogradely applied fluorescent dextran, medial glomeruli were devoid of Gₛₐᵣ-immunoreactivity (Fig. 4C–E). These included the posterior radial glomerulus, the glomerulus facing the anterior commissure, the posterior medial glomerulus, and individual fibers extending into the neuropil. The anterograde labeling shows directly that OSN axons extend to the medial glomeruli of the olfactory bulb, and that these axons do not stain by Gₛₐᵣ-immunocytochemistry. The anterograde labeling was limited to the OSN axons that took up the dextran when it was applied to the nasal cavity in vivo, and labeled OSN axons were less populous than with GS1B₄ lectin labeling (Fig. 4E). The lectin, which reacts with carbohydrate residues on extracellular axonal surface, stained more robustly than the dextran, which was confined to the cytoplasm within the narrow intracellular axonal space.

**Ventral ring and ventral cluster.** Glomeruli in this region were Gₛₐᵣ-IR (Fig. 4F,G).

**GloMerular subsets with differing Gₛₐᵣ expression persisted in the adult sea lamprey.** Medial glomeruli maintained an absence of Gₛₐᵣ-IR compared to the remaining glomerular units (Fig. 4H–I).

**Spatial relationship between 5HT-IR fibers and olfactory glomeruli.** The dorsal region of the olfactory bulb contained more robust 5HT-IR than the ventral region (Fig. 5). Double-
labeling experiments from eight larvae showed a constant pattern of 5HT innervation of glomerular territories.

**Dorsal cluster and dorsal ring.** Serotonergic fibers entered the glomerular units and were located between the closely spaced glomeruli (Fig. 5A).

**Anterior plexus and lateral chain.** Serotonergic fibers were present in the space between the anterior plexus and the lateral chain and surrounding the periphery of the anterior plexus and the lateral chain (Fig. 6B). As previously observed, 5HT-IR fibers extended along the...
olfactory nerve into the olfactory bulb (Zielinski et al., 2000). In the lateral portion of this region, 5HT-IR fibers extended into the olfactory bulb neuropil through the anterior plexus.

**Medial glomeruli.** Serotonergic fibers extended into the medial posterior glomerulus from the medial region of the olfactory nerve (Fig. 5C,D). Two medial glomeruli, the radiomedial glomeruli and the glomerulus adjacent to the anterior commissure, were devoid of 5HT-IR.

**Ventral ring and ventral cluster.** Glomerular modules in this region of the olfactory bulb did not contain 5HT-IR fibers (Fig. 5E).

In summary, 5HT-IR fibers and OSN axons were in close proximity in the dorsal cluster and the medial posterior glomerulus, and 5HT-immunoreactive fibers were least populous in the ventral olfactory bulb.

**DISCUSSION**

Examination of the distribution of substances that may be relevant to OSN function in the sea lamprey revealed spatially conserved glomerular associations. The olfactory receptor linked protein G_{olf} was expressed by sea lamprey OSNs projecting to dorsal, anterior, lateral, and ventral subsets. Innervation by nonolfactory 5HT-IR fibers was concentrated in the dorsal cluster and the medial posterior glomerulus. The principle differences between the glomerular groups is summarized in Table 1 and in Figure 6. Although these lampreys were from naturally occurring populations that represented diverse gene pools, there was remarkable consistency in these morphological, biochemical, and spatial characteristics.

**Bulbar G_{olf}-IR and OSN subtypes**

The molecular weight of the lamprey G_{olf}, 45 kDa, is identical to that of G_{olf} that regulates adenyl cyclase activity in mammals (Jones and Reed, 1989) and teleosts (Abogadie et al., 1995; Dellacorte et al., 1996). The ciliary localization of lamprey G_{olf} shown both through Western immunoblotting and immunocytochemically, supports the involvement of G_{olf} in olfactory sensory transduction. Therefore, this G-protein may be present in vertebrate ancestors over 400 million years ago, or it may have evolved in parallel during agnathan evolution. G_{olf} labeling was prominent in the glomerular units of the dorsal ring, anterior plexus, lateral chain, and ventral cluster. This is somewhat in contrast with prior reports in the rodent where G_{olf} was not detected in the olfactory bulb, although other molecules, including olfactory marker protein (Danciger et al., 1989) and mRNA for the olfactory marker protein (Wensley et al., 1995) and odor receptor are present in OSN axons (Mombaerts, 1996). This difference may underscore a species-specific phenotype but nevertheless emphasizes that subsets of lamprey OSNs may use G_{olf} transduction cascades. Detection in the axons may reflect further on the importance of G_{olf} in not only odor transduction, but also modulation of growth cone behavior through cascades that involve G_{olf} and cyclic nucleotide-gated channels, as suggested by Kafitz et al. (2000). The absence of G_{olf}-IR in the lamprey medial glomeruli points to a differing signal transduction mechanism for these compared to the other glomerular groupings. In various teleosts the medial olfactory path is associated with bile acid and steroid perception (e.g., Thommesen, 1978; Hara and Zhang, 1998), and in the zebrafish, medial glomeruli respond to chemostimulation by these compounds (Friedrich and Korschning, 1998). The OSN medial glomeruli in the lamprey may also constitute a subtype of OSN specialist expression. It is not surprising to find a subpopulation of lamprey OSNs without G_{olf}IR. The expression of various G-proteins in OSN subtypes appears to be a principle of the vertebrate olfactory system (e.g., Shinohara et al., 1992; Berghard and Buck, 1996; Jia and Halpern, 1996; Wekesa and Anholt, 1999; Hansen et al., 2001; Mezler et al., 2001). The medial location of the glomeruli that do not express G_{olf} implies that the OSNs projecting to these glomeruli use an alternate G-protein during olfactory sensory transduction. In tetrapods, chemoreceptive neurons of the vomerosal system contain the G-proteins G_{i}, G_{s}, (Halpern et al., 1995; Berghard and Buck, 1996; Jia and Halpern, 1996), and in the mam-
malian olfactory system, “necklace olfactory glomeruli” adjacent to the accessory olfactory bulb contain cGMP-stimulated phosphodiesterase and guanylyl cyclase-D (Juilfs et al., 1997) and heterogenous immunoreactivity for neural proteins (Ring et al., 1997). Therefore, the use of G-protein subtypes may have preceded the gnathostome radiation, or have evolved in parallel in gnathostome and agnathostome vertebrates.

Nonolfactory fibers adjacent to dorsal and medial glomeruli

In the lamprey olfactory bulb 5HT-IR fibers were located between glomerular groupings, particularly in lateral and medial locations of the dorsal hemisphere. In mammals, periglomerular serotonergic fibers originate from raphe nuclei (McLean and Shipley, 1987; Phulpot et al., 1994). However, in the lamprey the nonolfactory 5HT-IR fibers from the primary olfactory pathway enter the olfactory bulb (Zielinski et al., 2000). The present study extends previous results by investigating the spatial relationship between these fibers and olfactory glomeruli. The most prominent glomerular sites with 5HT innervation were the dorsal cluster and the medio-posterior glomeruli. This anterior dorsal/posterior medial ventral distribution is reminiscent of the dorsomedial pathway of the terminal nerve in the African lungfish (Von Bartheld and Mayer, 1988; Schober et al., 1994) and may indicate that this 5HT-immunoreactive pathway is a derivative of the nasal terminal nerve.

Spatial pattern of OSN projections

The pattern of glomerular organization in the larval lamprey was similar yet considerably reduced from the pattern formed by 18 glomerular groups in the olfactory bulb of the teleost Danio rerio (Baier and Korsching, 1994). In both, glomeruli were clustered in dorsal and ventral regions, the anterior plexus stretched from the dorsal to the ventral olfactory bulb, and the lateral group appeared as a chain of glomeruli. The overall simplicity of the lamprey’s glomerular arrangement can be seen from the ring of glomeruli encircling the dorsal and ventral regions. Images from previous studies of the adult silver lamprey Ichthyomyzon unicuspis, (Northcutt and Puzdrowski, 1988) and larval Petromyzon marinus (Tobet et al., 1996) have shown this ring-like pattern, as well as the medially located glomeruli. The four medial glomeruli (radial anterior, radial posterior, adjacent to the anterior commissure, and posterior medial) that we observed in the larval lamprey are fewer than the several medial glomeruli located in the olfactory bulb of the zebrafish (Baier and Korsching, 1994). The scarcity of glomeruli in the lamprey’s dorsomedial region of olfactory bulb may be due to the fact that this neuropil is occupied by contralateral secondary olfactory projections (Northcutt and Puzdrowski, 1988). The larval olfactory glomeruli were smaller than the 140 μm (average) diameter reported for glomeruli in adult Lampetra japonica (Iwahori et al., 1987), and within the range observed in adult zebrafish, (25 and 140 μm; Baier and Korsching, 1994) and rats (50–100 μm; Pinching and Powell, 1971). It is not surprising that the glomerular size is larger in adult lampreys than in larvae, as the olfactory epithelial surface area and the diameter of the olfactory nerve fascicles also increase during metamorphosis (Vandenbossche et al., 1997).

In conclusion, there are spatially conserved glomerular territories and substances that may be relevant to OSN activity which are distributed in a consistent manner. Serotonin-IR fibers are adjacent to specific glomerular modules and the G-protein G_{sR} is expressed by lamprey OSNs; however, a subpopulation of OSNs do not express G_{sR}. These data are the first to show that expression of G_{sR}, the GTP-binding protein linked to olfactory receptors, is present at the base of gnathostome radiation.

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LITERATURE CITED

Hildebrand JG, Shepherd GM. 1997. Mechanisms of olfactory discrimina-


