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 Golf, 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 Golf 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 GS1B4 lectin and by antero-grade 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 Golf-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, medial glomeruli, ventral ring, and ventral cluster. The dorsal, anterior, lateral, and ventral glomeruli contained olfactory sensory axon terminals that were Golf-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. © 2003 Wiley-Liss, Inc.

Indexing terms: Agnatha; olfactory sensory neurons; serotonin; Golf

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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 amphibians (Mezler 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 vomeronasal sensory neurons (Jia and Halpern, 1996) and in OSN subpopulations of amphibians (Mezler 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 (Jüllif 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 unicuspis* (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 leupeptid, 76.8 nM aprotinin, 0.7 μM pepstatin, 0.83 mM benzamidine, 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-Golf 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.

**GS1B₄ lectin histochemistry**

The staining of larval sea lamprey olfactory glomeruli by *Griffonia simplicifolia*-1 (GS-1 isolecitin B₄) was previously described (Tobet et al., 1996; Zielinski et al., 2000). Slides were incubated with biotin-conjugated lectin, *G. simplicifolia*-1 (GS-1 isolecitin B₄, Vector, Burlingame, CA; Fig. 2. Localization of G₄olf-IR in the olfactory epithelium. A and B show a double labeled preparation. A: Acetylated tubulin (AT)-immunolabeling in the larval nasal cavity shows intense labeling of the ciliary layer lining the luminal surface and of OSN soma in the olfactory epithelium (horizontal plane). Nonsensory epithelium is located on the right side of the micrograph. B: The luminal surface of the olfactory epithelium is G₄olf-IR, and the surface of the nonsensory epithelium is unlabeled (indicated by asterisks). OSN cilia, dendrites, cell bodies, and axons are G₄olf-IR (arrow). C: Obliquely sectioned OSNs show intense G₄olf-immunoreactivity in olfactory cilia. D: An OSN in a metamorphosing sea lamprey is intensely G₄olf-IR (Z-series of seven sections, taken at 0.2 μm steps. A, B, and D are the same magnification. Scale bar in B = 50 μm; 25 μm in C.)
Figure 3

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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 G_{olf} (1:100; 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 immunochemical 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, G_{olf} 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 and saved in TIFF format. The photomicroscope images were assembled into figures and labeled with Adobe PhotoShop (Mountain View, CA).

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 G_{olf} antibody. The molecular weight of G_{olf} 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 G_{olf} immunoreactive (IR) band in the ciliary fraction is supported through immunolocalization. Ciliary localization was seen in double-labeled preparations with acetylated tubulin and G_{olf} antibodies (Fig. 2A,B). Acetylated tubulin immunoreactivity indicated the presence of cilia (Fig. 2A). In larvae, the boundary between the olfactory epithelium and non-G_{olf}-expressing nasal epithelium was sharp and clear (Fig. 2B). High-power views confirmed strong G_{olf}-IR in cilia of OSNs (Fig. 2C). G_{olf} 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 G_{olf}-IR (Fig. 2D). The presence of G_{olf}-immunoreactivity in the OSN axons led us to examine spatial distribution of G_{olf}-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 bulb neuropil innervated by OSNs before chemically marking G_{olf}-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-
olfactory bulb. The area enclosed by a green box is shown at high power
immunolabeling, and yellow is colocalized Golf-immunolabeling and J:
merular region. Green is GS1B4 lectin histochemistry, red is Golf-
ritory of the glomerular groupings was consis-
ties in larval sea lampreys.

ty than the dextran, which was con-

ted to the OSN axons that took up the dextran when it

ted in the adult stage of the sea lamprey. Medial glo-


eruli maintained an absence of Golf-IR compared to the


ted in the larval stage of the sea lamprey, as previously reported (Tobet et al., 1996).

We consistently observed the following glomerular terr-

tories in larval sea lampreys.

**Dorsal cluster, 40–100 µm.** A cluster of 6–10 ola-

tory glomeruli was apparent close to the dorsal edge of the ola-


tory 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
dges 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 indi-
cated 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 extend-


ging 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 Kors-
sching, 1994).

**Medial glomeruli, 350–575 µm.** At bulbar 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 glomer-
ulus 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 glomer-
uli 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).

**Golf-immunolabeling.** Glomerular terri-
tories in the dorsal, anterior, lateral, and ventral regions of
the olfactory bulb contained Golf-IR. However, the me-
dial glomeruli were not Golf-IR (Fig. 4). The Golf-
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
Golf-immunoreactivity (Fig. 4A).

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

**Medial glomeruli.** Golf-immunoreactivity was absent
from the region occupied by medial glomeruli. In prepara-
tions double-labeled with Golf immunocytochemistry and
anterogradely applied fluorescent dextran, medial glomer-
uli were devoid of Golf-immunoreactivity (Fig. 4C–E).
These included the posterior radial glomerulus, the glo-
merulus 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 Golf-
immunocytochemistry. The anterograde labeling was lim-
ited 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 res-
ides on extracellular axonal surface, stained more ro-
bustly than the dextran, which was confined to the cyto-
plasm within the narrow intracellular axonal space.

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

Glomerular subsets with differing Golf expression per-
sisted in the adult stage of the sea lamprey. Medial glo-
meruli maintained an absence of Golf-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-

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Fig. 4. Golf-IR in olfactory glomeruli of the larval and adult lamprey. Scale bar in A = 100 µm. The depth of each section is indicated. A, B, F, and G are single-labeled preparations (Golf-immunocytochemistry). C and D are double-labeled (Golf-immunocytochemistry and fluorescent dextran). E is double-labeled (Golf-immunocytochemistry and GS1B4 lectin). A: 200 µm. Dorsal ring. B: 300 µm. Anterior plexus and lateral chain. C: 350 µm and D: 400 µm. Anterior plexus, lateral chain, and medial glomeruli. In C the medial edge of the olfactory bulb is located on the right edge of the micrograph. In D the center of the micrograph shows the medial regions of adjacent olfactory bulbs. The anterior and lateral glomeruli show double-labeling for Golf and anterogradely applied fluo-

rescent dextran. These labels do not show complete colocalization, as the green (dextran) is within the cytoplasm of the OSN axons and the red Golf is localized on the membranous surface. OSN axons in the medial glo-
merulus facing the anterior commissure (AC) labeled with the dextran, but not with Golf-immunocytochemistry. E: 500 µm. Ventral ring and medial glomeruli (Z series of eight sections taken at 1-µm intervals). The glomeruli of the ventral ring show double-labeling for Golf and for GS1B4 lectin. Both stains are localized on the cell membrane. The medial glo-
meruli, including posterior medial glomeruli (PM), are not Golf-IR. F: 600 µm. Ventral ring. G: 700 µm. Ventral cluster. H to J show a horizontal view of the olfactory bulb from an adult sea lamprey, double-labeled, GS1B4 lectin histochemistry, and Golf-immunocytochemistry. H and I are fluorescence micrographs shown at the same magnification. Scale bar in

H = 500 µm. I: GS1B4 lectin stains olfactory glomeruli in the anterior, lateral, and medial regions. I: Anterior and lateral olfactory glomeruli are Golf-IR. The area enclosed by a green box is shown at high power in J: A high-power confocal micrograph of the medial olfactory glomerular region. Green is GS1B4 lectin histochemistry, red is Golf-immunolabeling, and yellow is colocalized Golf-immunolabeling and GS1B4 lectin staining. The red and green channels were imaged in series to avoid fluorescent bleed-through on the red channel. Scale bar = 25 µm.
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 glomerular 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 G$_{olf}$, shown both through Western immunoblotting and immunocytochemically, supports the involvement of G$_{olf}$ in olfactory sensory transduction. Therefore, this G-protein may have been 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 cluster, 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 pathway 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., Shinozaka et al., 1992; Berghard and Buck, 1996; Jia and Halpern, 1996; Weksel 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.
malian olfactory system, “necklace olfactory glomeruli” adjacent to the accessory olfactory bulb contain cGMP-stimulated phosphodiesterase and guanylyl cyclase-D (Julifs et al., 1997) and heterogeneous 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 at 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; Philpot 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 glomerulus. This anterior dorsal/posterior medial ventral distribution is reminiscent of the dorsomedial pathway of the terminal nerve in the African lungfish (Von Barfeldt 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 Korschling, 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 Korschling, 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 Lamproptera japonica (Iwahori et al., 1987), and within the range observed in adult zebrafish (25 and 140 μm; Baier and Korschling, 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 Goα is expressed by lamprey OSNs. However, a subpopulation of OSNs do not express Goα. These data are the first to show that expression of Goα, the GTP-binding protein linked to olfactory receptors, is present at the base of gnathostome radiation.

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