GREAT LAKES FISHERY COMMISSION

2004 Project Completion Report[1]

The Analysis of Pheromone Identification by Sea Lamprey through Functional Imaging of Olfactory Glomeruli

by:

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Title: THE ANALYSIS OF PHEROMONE IDENTIFICATION BY SEA LAMPREY THROUGH FUNCTIONAL IMAGING OF OLFACTORY GLOMERULI

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End Date: Dec. 31, 2003

PROJECT DESCRIPTION

This project will develop a dynamic optical imaging technique for assessing the ability of sea lampreys to recognize pheromones. Furthermore, we will investigate temporal, spatial and response magnitude differences during the sea lamprey life cycle. By identifying the glomerular location(s) in the olfactory bulb that respond to pheromone stimulation, the potency of pheromones on specific stages can be determined.

PROJECT OBJECTIVES:

The objective of this preliminary study is to identify sea lamprey glomerular units that respond to olfactory stimulation by pheromones. The research objectives include the following:

1. The localization of glomerular responses to the migratory pheromone and to the reproductive pheromone.
2. Dose-response curves of glomerular activity following the application of the pheromones.
3. A developmental survey of glomerular responses to the identified pheromones.

Reviewer's Suggestions:

The comments from the reviewers of this pilot project voiced concerns about the location of olfactory sensory neurons that innervate the specific glomerular territories.

Presentations:

Arbuckle, W.J., Firby, A.E., Zielinski, B.S. The spatial division of the olfactory system of the larval sea lamprey (Petromyzon Marinus) based on the expression of a G protein, G_{olf}, in the olfactory sensory neurons and olfactory bulb glomeruli. Association for Chemoreception Sciences 2004.

W.J. Arbuckle, A.E. Firby and Zielinski, B.S., Rhinotopy in the larval stage of the sea lamprey, *Petromyzon marinus, Association for Chemoreception Sciences,* Sarasota, FL April 9-13, 2003.

M.Sc. Thesis: Biochemical and Spatial Studies of Pheromone Synthesis and Chemoreception. W.J. Arbuckle. In Progress

B.Sc. Thesis: A.E. Firby Rhinotopy in the larval stage of the sea lamprey, *Petromyzon marinus,*

Expenditures: Stipend for student assistance (Ms. Ashley Firby, Mr. Wes Arbuckle). Supplies (antibodies, reagents, fluorogenic dyes, dissection instruments), wet lab supplies, travel for electro-encephalogram training to Freshwater Institute (Dr. T.J. Hara); microscope fees and upkeep.

Task 1:

The objective of this pilot project was to observe the brain (olfactory bulb) location of pheromone responses through functional imaging of an *in vitro* preparation. The nasal location of the olfactory sensory neurons was investigated to make sure that odorants are applied to the appropriate nasal space in the imaging preparation. Our previous studies showed that olfactory sensory neuron terminals (glomeruli) that are located medially in the olfactory bulb, are biochemically distinct from the remaining glomerular territories (Frontini et al., 2003, and Fig. 1 in this report). We sought to understand the spatial nasal (olfactory epithelial) origin of olfactory sensory neurons that innervate the glomerular territories that will be analyzed. We injected individual glomerular territories with fluorescent dextran dyes in brain/nasal explants, and allowed the dye to fill the injected cells over 20 hours (fig. 2). The results of this study show that medial glomeruli receive olfactory sensory neuron projections from the ventral hemisphere of olfactory epithelium in the larval lamprey (Fig. 3 – 5). Glomeruli in the dorsal region of olfactory bulb were innervated by olfactory sensory neurons located more broadly in the peripheral olfactory organ (Fig. 6 - 7). This information is essential for developing the optical imaging preparation.

Figure 1. A triple labeled confocal image of a horizontal section from the mid region of the olfactory bulb shows a medial glomerulus (arrows) with olfactory sensory neurons that do not contain the protein G_{olf}, whereas the anterior and lateral glomeruli (puple/pink) contain this protein.

Green: Cells micro-injected with fluorescent green dextran
Red: olfactory sensory neurons labeled with GS1B4 lectin, no G_{olf}-immunoreactivity
Purple/pink: olfactory sensory neurons with G_{olf}-immunoreactivity
Yellow: olfactory sensory neurons (GS1B4 lectin labeling) that were injected with fluorescent green, and were not G_{olf}-immunoreactive

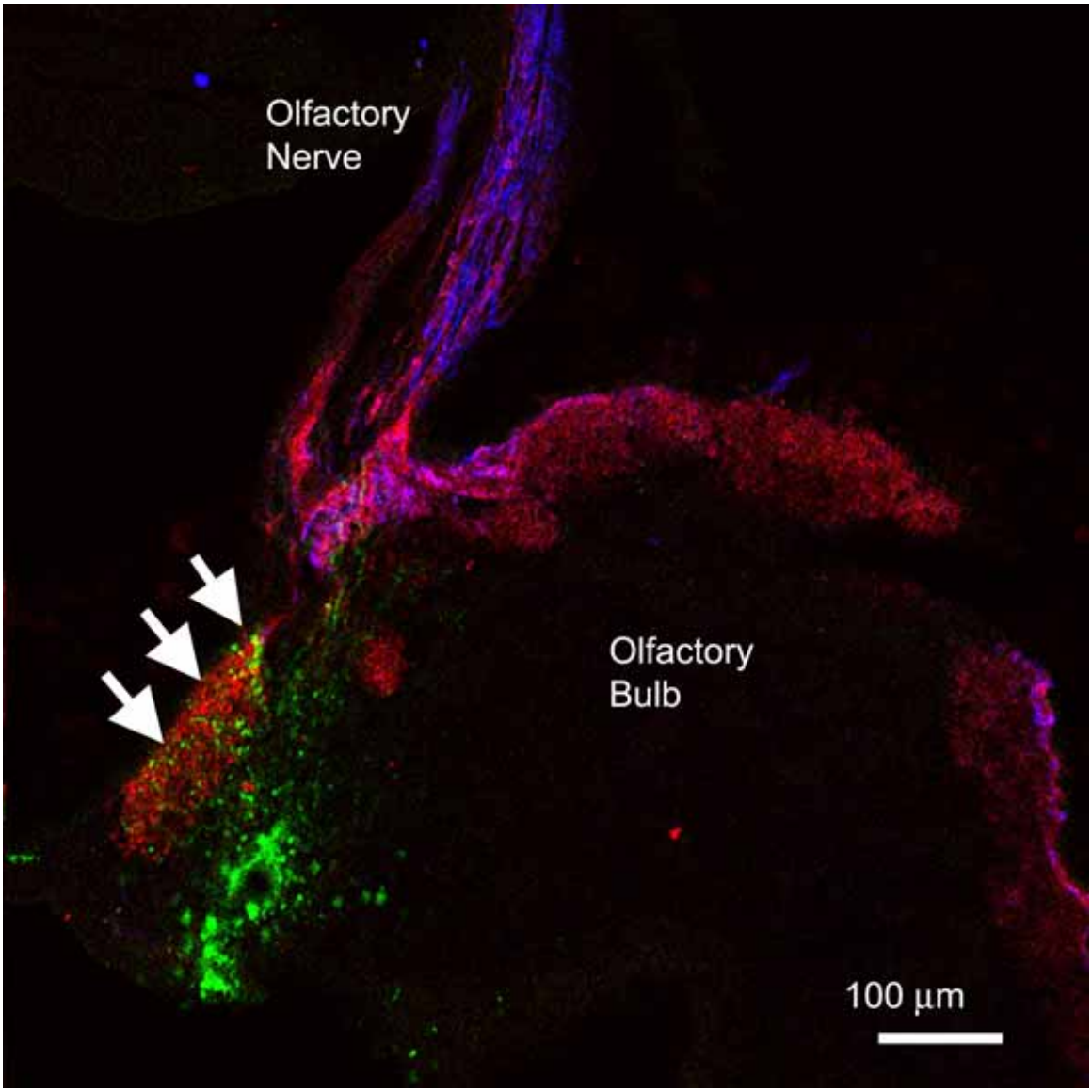
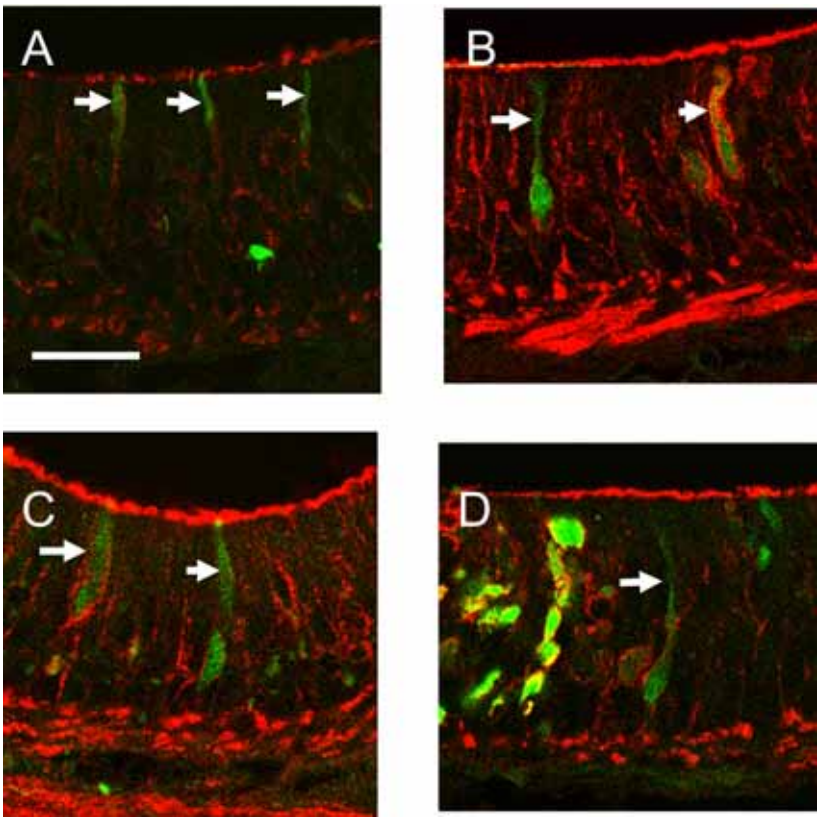


Figure 2. Confocal images of the olfactory epithelium with retrogradely labeled olfactory sensory neurons, from injections that included medial glomeruli. The green cells are filled with dye that was injected into specific glomerular territories in the olfactory bulb. The red labeling shows G_{olf}-immunoreactivity. Green cells that are outlined in red are G_{olf}-immunoreactive olfactory sensory neurons. Green cells that are not outlined in red are not G_{olf}-immunoreactive. Olfactory sensory neurons are recognized by narrow dendrites (arrows) and balloon-like cell bodies. Some dextran labeled cells did not appear to have dendrites (cells on the left side of panel D). Micrometer bar is 20 μm.



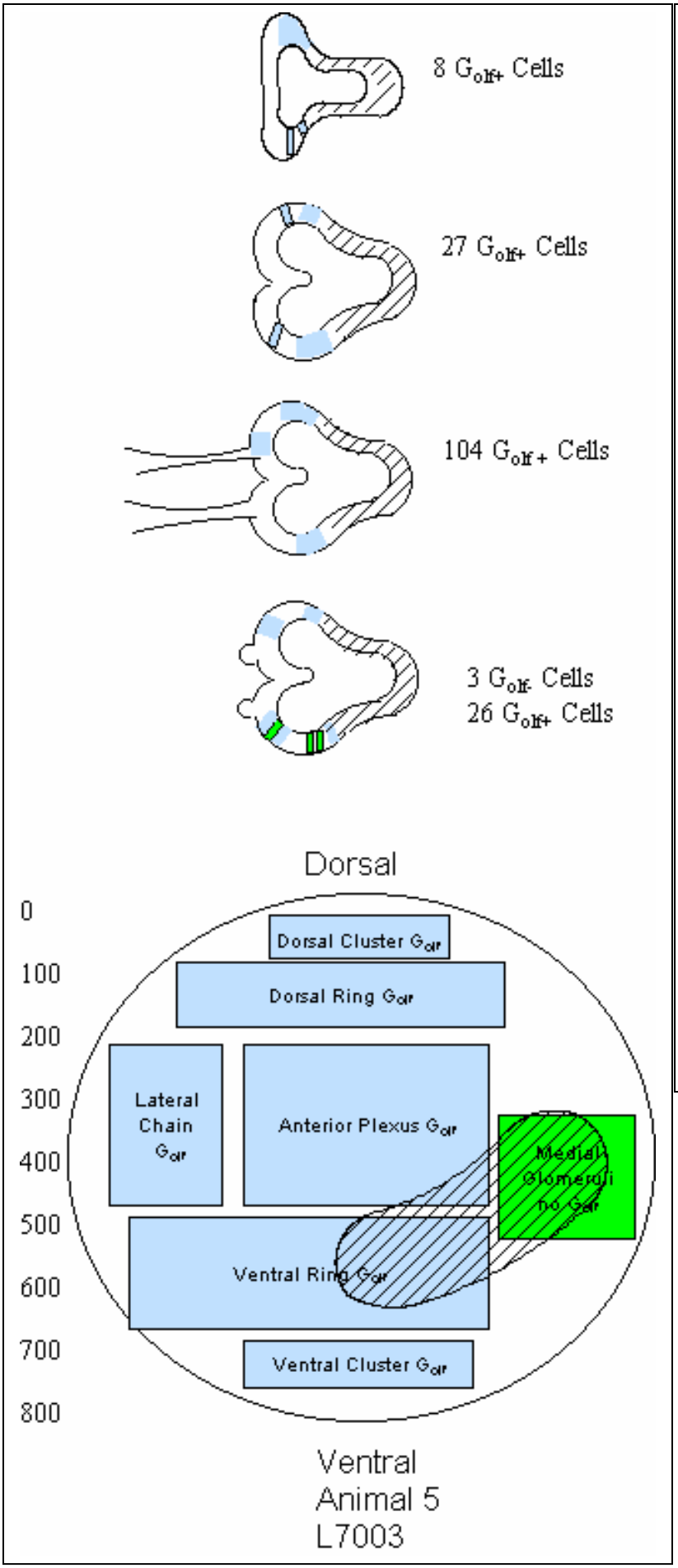


Fig. 3
The Location of Retrogradely Labeled Olfactory Sensory Neurons:

Dorsal Olfactory Epithelium

Mid Olfactory Epithelium

Ventral Olfactory Epithelium

Olfactory bulb:
Blue – glomeruli with Golf-IR

Green – glomeruli with no Golf-IR

Diagonal Lines: location of dextran injection.

Figure 4. The Location of Retrogradely Labeled Olfactory Sensory Neurons:
Blue – glomeruli with Golf-IR

Green – glomeruli with no Golf-IR

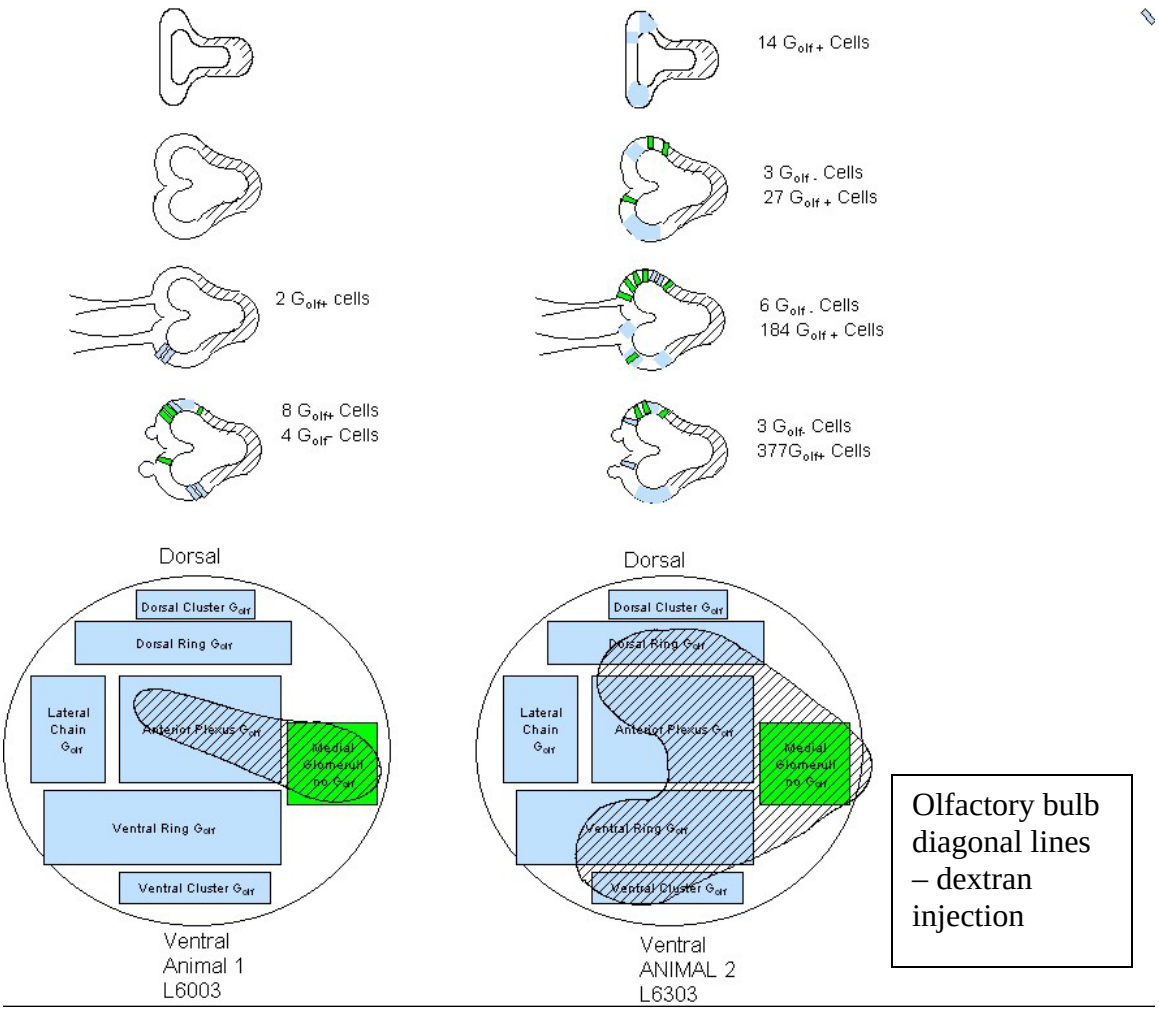


Figure 5. The Location of Retrogradely Labeled Olfactory Sensory Neurons:
Blue – glomeruli with Golf-IR
Green – glomeruli with no Golf-IR

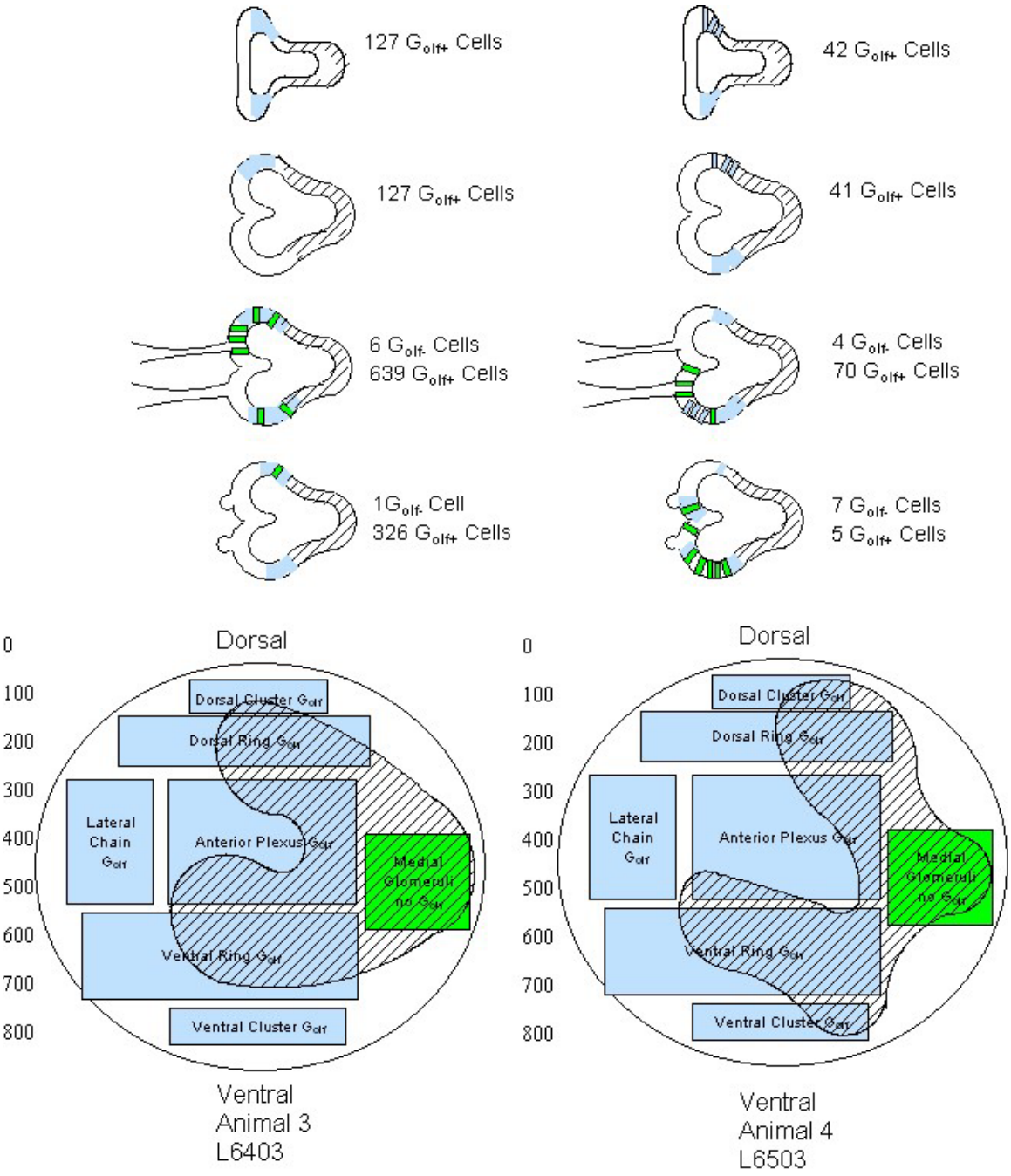
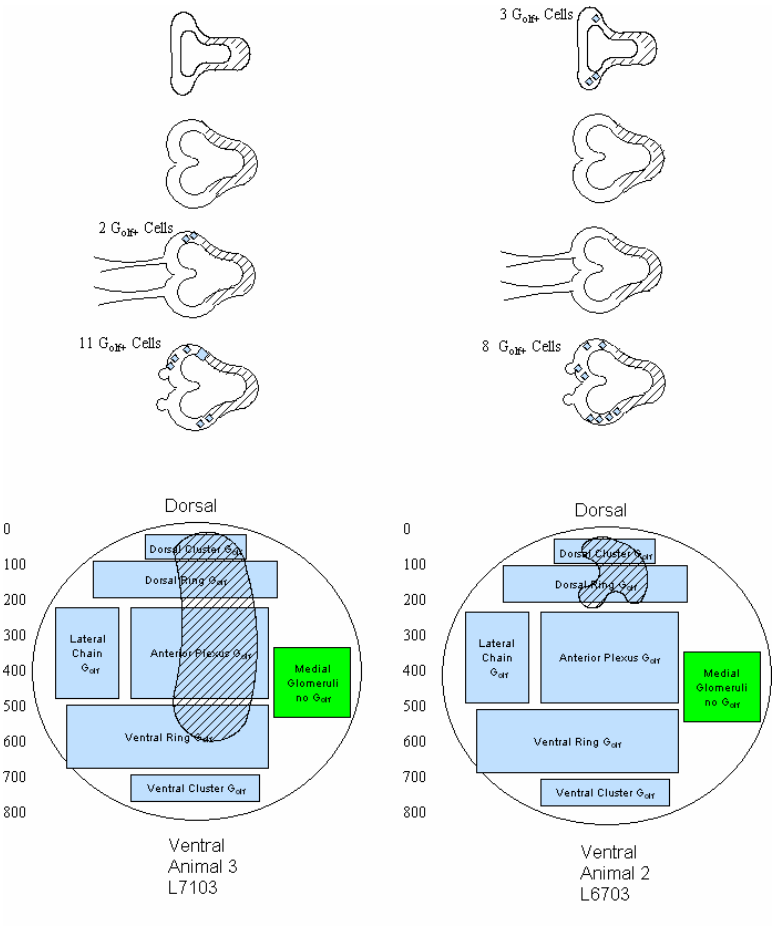


Figure 6. The Location of Retrogradely Labeled Olfactory Sensory Neurons:
Blue – glomeruli with Golf-IR
Green – glomeruli with no Golf-IR



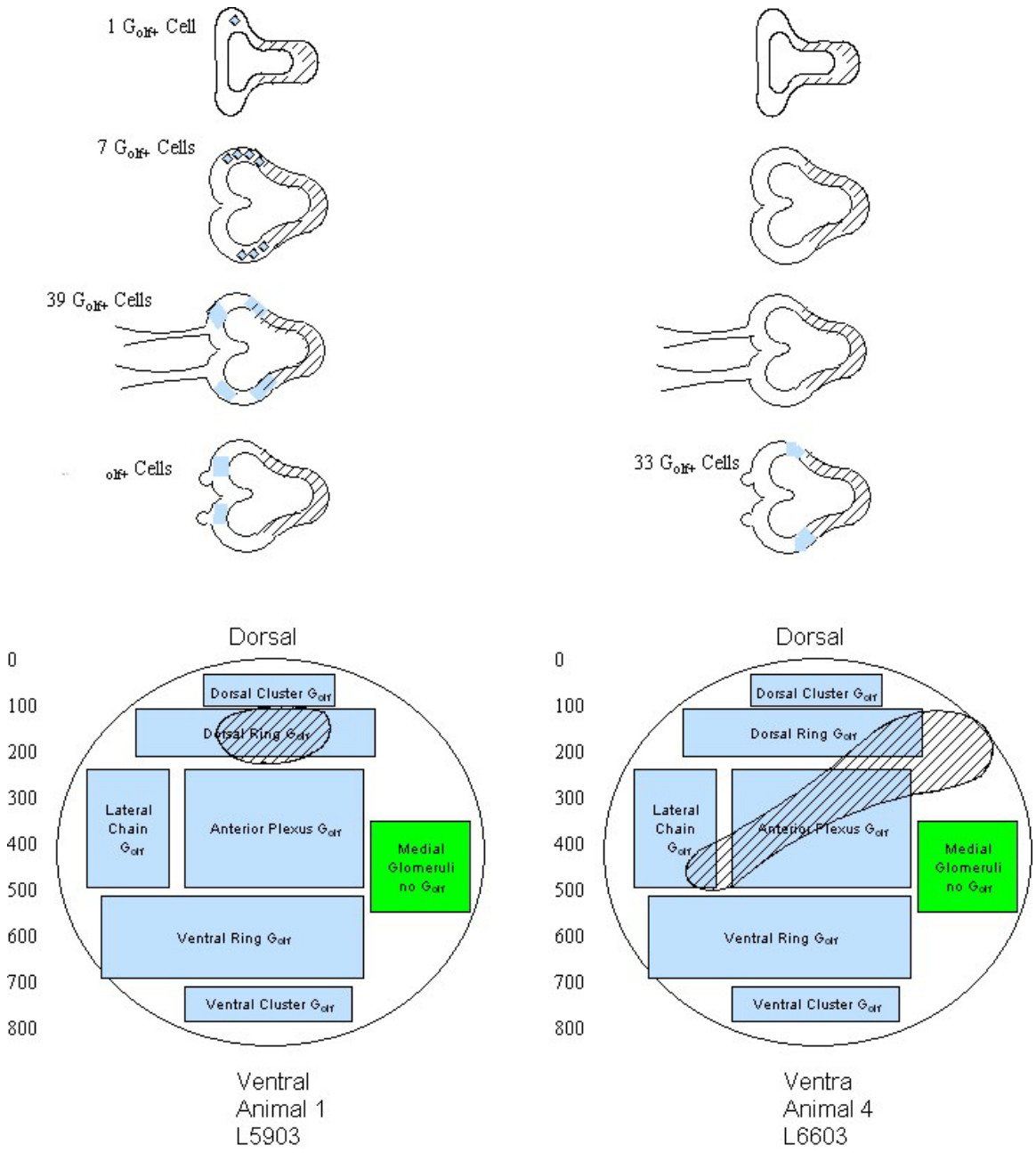


Figure 7 . The Location of Retrogradely Labeled Olfactory Sensory Neurons:
Blue – glomeruli with Golf-IR
Green – glomeruli with no Golf-IR
Diagonal Lines in olfactory bulb: location of dextran injection.

Task 2. We are addressing technical difficulties with obtaining calcium signals from the dynamic imaging technique. These are being addressed at three levels: 1. Assessing spatial activity through FOSb antibody localization (Chung-Davidson et al., 2004). We have started this study during the past two months. 2. Examining the functional integrity of the olfactory epithelium, and 3. Electrophysiological recording in the olfactory bulb to determine the responsiveness of this tissue in the *in vitro* preparation. The last two tests will be conducted in 2004.

Spatial Assessment of olfactory bulb activity through FOSb immunocytochemistry.

When cells have been metabolically and functionally active, specific proteins, known as early gene expression molecules, are formed. The appearance of these proteins can show the location of these active cells. Recently, Chung-Davidson et al. (2004) have adapted this technique for use in the sea lamprey. We report our test of this FOSb expression in the larval sea lamprey olfactory bulb, following exposure to the odorant L-arginine. Test with the reproductive pheromone is in progress. In this study, the odorant was pumped into a tank with larvae. Larvae in a second tank received untreated water. Our initial study comprised of three odorant (1 μm L-arginine), and three untreated larvae. These preliminary data suggest that this odorant stimulates FOSb production in specific dorsal, mid and ventral foci of the olfactory bulb (fig. 8). We are currently repeating these tests.

GENERAL CONCLUSION:

This preliminary study lays the groundwork for spatial-temporal study of pheromone responses in the sea lamprey. A complete understanding of how pheromones stimulate biological activity (e.g. movement and reproductive activity), will lead to comprehensive use of pheromones for population management.

Literature Cited:

Chung-Davidson, Y-W; Yun S-S; Teeter J, Li W. Brain Pathways and Behavioral Responses to Weak Electric Fields in Parasitic Sea Lampreys (*Petromyzon marinus*) Behavioral Neuroscience (in press).

Frontini A, Zaidi AU, Hua H, Wolak TP, Greer CA, Kafitz KA, Li W, Zielinski B. Glomerular Territories in the Olfactory Bulb from the Larval Stage of the Sea Lamprey *Petromyzon marinus:* Journal of Comparative Neurology 465, 27-37.

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Fig 8. FOSb immunoreactivity in horizontal sections of the olfactory bulb and telencephalon from larval sea lampreys. The left panel was taken from larvae exposed to 1 μm L-arginine, and the right panel shows tissue from larvae exposed to untreated water. These are representative sections from dorsal, mid and ventral regions. The arrows point to FOS0immunoreactive neuropil.