The Ultrastructure of the Olfactory System in Two Species of Short-Tailed Shrews, *Blarina brevicauda* and *Blarina carolinensis*

Lisa Johnson Byrum

*Old Dominion University*

Follow this and additional works at: [https://digitalcommons.odu.edu/biomedicalsciences_etds](https://digitalcommons.odu.edu/biomedicalsciences_etds)

Part of the Animal Structures Commons, Cell Biology Commons, and the Neurology Commons

**Recommended Citation**

Byrum, Lisa J.. "The Ultrastructure of the Olfactory System in Two Species of Short-Tailed Shrews, *Blarina brevicauda* and *Blarina carolinensis*" (2004). Doctor of Philosophy (PhD), Dissertation, , Old Dominion University, DOI: 10.25777/5kav-3e84

[https://digitalcommons.odu.edu/biomedicalsciences_etds/12](https://digitalcommons.odu.edu/biomedicalsciences_etds/12)

This Dissertation is brought to you for free and open access by the College of Sciences at ODU Digital Commons. It has been accepted for inclusion in Theses and Dissertations in Biomedical Sciences by an authorized administrator of ODU Digital Commons. For more information, please contact digitalcommons@odu.edu.
THE ULTRASTRUCTURE OF THE OLFACTORY SYSTEM IN
TWO SPECIES OF SHORT-TAILED SHREWS, BLARINA
BREVICAUDA AND BLARINA CAROLINENSIS

by

Lisa Johnson Byrum
B.S. December 1986, Old Dominion University
M.S. May 1995, Old Dominion University

A Dissertation Submitted to the Faculty of
Old Dominion University in Partial Fulfillment of the
Requirement for the Degree of

DOCTOR OF PHILOSOPHY

BIOMEDICAL SCIENCES

OLD DOMINION UNIVERSITY
August 2004

Approved by:

Keith Carson (Director)

Alan Savitzky (Member)

Charles Morgan (Member)

David Scott (Member)
ABSTRACT

THE ULTRASTRUCTURE OF THE OLFACTORY SYSTEM IN TWO SPECIES OF SHORT-TAILED SHREWS, BLARINA BREVICAUDA AND BLARINA CAROLINENSIS

Lisa Johnson Byrum
Old Dominion University, 2004
Director: Dr. Keith Carson

Several studies of the fine structure of the olfactory system of rodents have been conducted, but very little research has been done on members of the Insectivora. The olfactory systems of the northern short-tailed shrew, *Blarina brevicauda*, and the southern short-tailed shrew, *Blarina carolinensis*, were examined by light and electron microscopy. These shrews were live trapped in the vicinity of Norfolk, Virginia throughout all months of the year. Olfactory tissues were processed following standard transmission and scanning electron microscopy protocols. The olfactory system structures investigated included the olfactory epithelium/mucosa (OEM), main olfactory bulb (MOB), accessory olfactory bulb (AOB), anterior olfactory nucleus (AON), olfactory tubercle (OT), and piriform cortex (PC). A new type of supporting cell, the light supporting cell, was observed in the OEM in *Blarina*. The MOB and AOB were laminated structures that were similar to other macrosmatic mammals. The AON was divided into typical mammalian subdivisions but the AON appeared at a significantly more rostral level within the MOB compared to other mammals. The AON was displaced in a dorsolateral direction in shrews. The trilaminar OT and PC had smaller cells compared to other small mammals. Light and electron microscopic data show that
the olfactory system in *Blarina* differed from other small macrosmatic mammals and was well-developed.
This dissertation is dedicated to my encourager, my comforter, and my dearest friend.
ACKNOWLEDGMENTS

I am appreciative for the counsel and direction from my dissertation committee members, Drs. Alan Savitzky, Charles Morgan, and David Scott whose wisdom was indispensable in organizing and conducting this research project. My major professor, Dr. Keith Carson, generously shared his expertise and knowledge throughout this program. His persistent leadership and instruction were crucial to the successful completion of this project and I am deeply grateful to him. Mike Adam and Denise Sliter enthusiastically shared their knowledge of electron microscopy with me, offering many hours of training and coaching.

I am thankful for the support from my family and friends. From my mother I learned of the value of education; my father illustrated humbleness that must accompany an education. I have been blessed with honorable friends whose camaraderie continually elevated my spirits. Their confidence in me has been an essential factor in my achievements.

And finally, I must acknowledge my husband, Frank. Helen Keller is attributed with the words “One can never consent to creep when one feels an impulse to soar.” Because of Frank, I will never be the same again, for I have learned to soar. He has been an infinite source of encouragement, inspiration, hope, and optimism for me. Without him, I scarcely could creep. His faith in me has been and continues to be a priceless treasure. Frank remains my strongest ally, my loyal advocate, and my steadfast friend. The years of his commitment and the source of my strength have been my solid anchor.
# TABLE OF CONTENTS

| LIST OF TABLES ................................................................. | ix |
| LIST OF FIGURES ............................................................... | x |

## CHAPTER

### I. INTRODUCTION ......................................................................................... 1
   1.1 The Order Insectivora .............................................................. 1
   1.2 The Significant of Sensory Systems ........................................ 2
   1.3 The Importance of Olfaction .................................................. 3
   1.4 The Statement of the Problem ............................................... 4
   1.5 Olfactory Epithelium and Mucosa (OEM) ................................. 4
   1.6 Main Olfactory Bulb (MOB) .................................................. 6
   1.7 Accessory Olfactory Bulb (AOB) ......................................... 7
   1.8 Anterior Olfactory Nucleus (AON) ...................................... 9
   1.9 Olfactory Tubercle (OT) ....................................................... 9
   1.10 Piriform Cortex (PC) ......................................................... 11

### II. METHODS ................................................................................................. 13
   2.1 Introduction ........................................................................... 13
   2.2 Light Microscopy of Epoxy-Embedded Tissues ...................... 13
   2.3 Transmission Electron Microscopy of Epoxy-Embedded Tissues 14
   2.4 Scanning Electron Microscopy of Olfactory Epithelium Tissues 15
   2.5 Light Microscopic Histochemistry of Frozen Sections .......... 15
   2.6 Light Microscopic Histochemistry of Paraffin-Embedded Tissues 16
   2.7 Table of Abbreviations ......................................................... 16

### III. OLFACTORY EPITHELIUM AND MUCOSA ........................................... 18
   3.1 Introduction ........................................................................... 18
   3.2 Results ................................................................................... 18
   3.3 Discussion .............................................................................. 30
   3.4 Comparative Studies ......................................................... 34
   3.5 Conclusion ............................................................................. 34

### IV. MAIN OLFACTORY BULB .................................................................. 35
   4.1 Introduction ........................................................................... 35
   4.2 Results ................................................................................... 36
   4.3 Discussion .............................................................................. 63
   4.4 Comparative Studies ......................................................... 75
   4.5 Conclusion ............................................................................. 77

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Abbreviations</td>
<td>17</td>
</tr>
<tr>
<td>2. Glomerular diameter range</td>
<td>65</td>
</tr>
<tr>
<td>3. Synaptic patterns of the glomerular layer</td>
<td>66</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1.</td>
<td>Light micrograph of the olfactory epithelium</td>
</tr>
<tr>
<td>2.</td>
<td>Light micrograph of the five cell types of the olfactory epithelium</td>
</tr>
<tr>
<td>3.</td>
<td>Light micrograph of the axon bundle of the lamina propria</td>
</tr>
<tr>
<td>4.</td>
<td>TEM micrograph of the receptor cell of the olfactory epithelium</td>
</tr>
<tr>
<td>5.</td>
<td>TEM micrograph of the receptor cell dendrite</td>
</tr>
<tr>
<td>6.</td>
<td>TEM micrograph of the cilium of the receptor cell</td>
</tr>
<tr>
<td>7.</td>
<td>SEM micrograph of the dense carpet of cilia</td>
</tr>
<tr>
<td>8.</td>
<td>TEM micrograph of the microtubule arrangement</td>
</tr>
<tr>
<td>9.</td>
<td>TEM micrograph of two types of supporting cells</td>
</tr>
<tr>
<td>10.</td>
<td>TEM micrograph of two types of basal cells</td>
</tr>
<tr>
<td>11.</td>
<td>TEM micrograph of the axon bundle</td>
</tr>
<tr>
<td>12.</td>
<td>TEM micrograph of the Bowman's glands</td>
</tr>
<tr>
<td>13.</td>
<td>Line drawing of the lateral view of the cortex and cerebellum</td>
</tr>
<tr>
<td>14.</td>
<td>Light micrograph of the sagittal plane of the main olfactory bulb</td>
</tr>
<tr>
<td>15.</td>
<td>Light micrograph of the rostral main olfactory bulb</td>
</tr>
<tr>
<td>16.</td>
<td>Light micrograph of the mid-caudal main olfactory bulb</td>
</tr>
<tr>
<td>17.</td>
<td>Light micrograph of the caudal main olfactory bulb</td>
</tr>
<tr>
<td>18.</td>
<td>Light micrograph of the main olfactory bulb layers</td>
</tr>
<tr>
<td>19.</td>
<td>Light micrograph of the olfactory nerve layer</td>
</tr>
<tr>
<td>20.</td>
<td>Light micrograph of the glomerular layer</td>
</tr>
</tbody>
</table>
21. Light micrograph of the external tufted cell .................................................................44
22. Light micrograph of the external plexiform layer..........................................................44
23. Light micrograph of the mitral cell layer .....................................................................46
24. Light micrograph of the granule cell layer ...................................................................46
25. TEM micrograph of dark ensheathing cells .................................................................47
26. TEM micrograph of a light ensheathing cell ...............................................................47
27. TEM micrograph of periglomerular cells ...................................................................49
28. TEM micrograph of the short-axon cell ......................................................................50
29. TEM micrograph of the external tufted cell ...............................................................51
30. TEM micrograph of axons and dendrites in the glomeruli ..........................................52
31. TEM micrograph of a dendro-dendritic synapse .........................................................54
32. TEM micrograph of a reciprocal synapse ...................................................................54
33. TEM micrograph of an axo-dendritic synapse .............................................................55
34. TEM micrograph of a dendro-dendritic synapse ..........................................................55
35. TEM micrograph of dendro-dendritic synapses ...........................................................56
36. TEM micrograph of pleomorphic vesicles ..................................................................56
37. TEM micrograph of a myelinated neuron ...................................................................57
38. TEM micrograph of the thin myelin sheath .................................................................58
39. TEM micrograph of a myelinated dendrite .................................................................58
40. TEM micrograph of a mitral cell ..................................................................................60
41. TEM micrograph of myelinated axons ......................................................................61
42. TEM micrograph of the neuropil of the granule cell layer ...........................................61
43. TEM micrograph of dark and light granule cells .........................................................62
44. Light micrograph of the rostral accessory olfactory bulb .............................................80
45. Light micrograph of caudal accessory olfactory bulb ...................................................80
46. Light micrograph of the laminar organization of the accessory olfactory bulb ........81
47. Light micrograph of an overview of the accessory olfactory bulb ..............................83
48. Light micrograph of the vomeronasal nerve layer ........................................................83
49. Light micrograph of the glomerular layer ......................................................................84
50. Light micrograph of the external plexiform/mitral-tufted cell layer ............................84
51. Light micrograph of the granule cell layer .....................................................................86
52. TEM micrograph of vomeronasal nerve layer ...............................................................86
53. TEM micrograph of ensheathing cells of the vomeronasal nerve layer .......................87
54. TEM micrograph of a glomerulus ...................................................................................89
55. TEM micrograph of a axo-dendritic synapse ................................................................89
56. TEM micrograph of a dendro-dendritic synapse ..........................................................90
57. TEM micrograph of a dendro-dendritic synapse ..........................................................90
58. TEM micrograph of a periglomerular cell .....................................................................91
59. TEM micrograph of mitral/tufted cells ..........................................................................91
60. TEM micrograph of pleomorphic vesicles ....................................................................93
61. TEM micrograph of spherical vesicles ..........................................................................93
62. TEM micrograph of the internal plexiform layer ..........................................................94
63. TEM micrograph of the granule cell layer .....................................................................94
64. Light micrograph of the rostral pars lateralis of the anterior olfactory nucleus ..........108
65. Light micrograph of the rostral pars externa of the anterior olfactory nucleus ........108
66. Light micrograph of the mid-caudal pars externa ........................................................109

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
67. Light micrograph of the rostral pars dorsalis and pars ventralis............................109
68. Light micrograph of the rostral pars medialis..........................................................110
69. Light micrograph of the mid-caudal pars medialis................................................110
70. Light micrograph of the caudal pars medialis..........................................................112
71. Light micrograph of the pars posterior .................................................................112
72. Light micrograph of the olfactory ventricle and subventricular zone......................113
73. Light micrograph of ependymal cells ....................................................................113
74. Light micrograph of pyramidal cells of the pars lateralis........................................115
75. Light micrograph of the dorsal limb of the pars externa.........................................115
76. Light micrograph of the ventral limb of the pars externa.......................................116
77. Light micrograph of pyramidal cells of the pars dorsalis.........................................116
78. Light micrograph of pyramidal cells of the pars medialis........................................118
79. Light micrograph of pyramidal cells of the pars ventralis........................................118
80. Light micrograph of pyramidal cells of the pars posterior.......................................119
81. TEM micrograph of pars lateralis pyramidal cell with apical dendrite ..................119
82. TEM micrograph of synapses in the pars lateralis neuropil....................................121
83. TEM micrograph of pars externa pyramidal cell ....................................................121
84. TEM micrograph of synapses in the pars externa neuropil ....................................122
85. TEM micrograph of pars dorsalis pyramidal cell ...................................................122
86. Light micrograph of a sagittal plane of the olfactory tubercle...............................133
87. Light micrograph of the rostral olfactory tubercle..................................................134
88. Light micrograph of the mid-caudal olfactory tubercle..........................................134
89. Light micrograph of the caudal olfactory tubercle..................................................135
90. Light micrograph of the trilaminar olfactory tubercle................................................135
91. Light micrograph of the plexiform layer of the olfactory tubercle.................................137
92. Light micrograph of the pyramidal cell layer of the olfactory tubercle............................137
93. Light micrograph of the polymorph cell layer of the olfactory tubercle............................139
94. Light micrograph of granule cells of the Islands of Calleja.............................................139
95. Light micrograph of the rostral piriform cortex............................................................145
96. Light micrograph of the mid-caudal piriform cortex....................................................145
97. Light micrograph of the caudal piriform cortex............................................................146
98. Light micrograph of the trilaminar piriform cortex......................................................146
99. Light micrograph of Layer I of the piriform cortex......................................................148
100. Light micrograph of Layer II pyramidal cells of the piriform cortex...............................148
101. Light micrograph of pyramidal cell dendrites...............................................................150
102. Light micrograph of multipolar neurons of the piriform cortex.....................................150
CHAPTER I

INTRODUCTION

1.1 The Order Insectivora

Shrews make up greater than 70% of the Order Insectivora and are described as having considerable appetites with a correspondingly high metabolism. As predators, these small mammals feed mainly upon invertebrates. Shrews share the Order Insectivora with moles, hedgehogs, and tenrecs. The family Soricidae (shrews) is divided into two subfamilies: Soricinae and Crocidurinae (Catania et al., 1999). Shrews of the subfamily Soricinae have a reddish-brown coloration of the teeth due to iron deposits in the enamel and are known as the red-toothed shrews (Dotsch and Koenigswald, 1978, Larochelle and Baron, 1989b). Shrews (Soricidae) range from 2.5 grams to over 200 grams in body weight. Shrews are located on all major landmasses with the exception of Australia, New Zealand, Antarctica, Greenland, Iceland, and Tasmania. Generally, shrews are terrestrial mammals occupying woodlands, forests, and grasslands although several species of aquatic forms thrive in marshy and aquatic habitats (Churchfield, 1990; Nowak, 1999). As terrestrial insectivores, shrews have numerous morphological specializations that allow these small mammals to survive in different habitats. The cylindrical body, tail length, small eyes, and short pelage are examples of various specializations (Stephan et al., 1991; Baron and Stephan, 1996). Shrews remain active throughout all seasons of the year. Shrews are among the most primitive placental mammals, first evolving in the late Eocene/early Oligocene period, approximately 38 million years ago, some time after the...
dinosaurs disappeared. Shrews have changed little since their first appearance among the dinosaurs. Present-day Insectivora have retained many features of the earliest mammals (Churchfield, 1990). Therefore, the study of their brains may yield significant insight into the process of mammalian brain evolution.

1.2 The Significance of Sensory Systems

The visual system of the Insectivora is generally poorly developed compared to most other mammals. Shrews have the smallest eyes of the terrestrial Insectivora, measuring between 0.7 – 1.5 mm in diameter (Churchfield, 1990; Baron and Stephan, 1996; Stephan et al., 1991). It appears that vision functions merely to discriminate light intensity (Merriam, 1884; Branis, 1981). In agreement with previous researchers, Ryzen and Campbell (1955) reported that shrew visual cortex was thin and poorly developed. The small size of the optic nerve, the minute lateral geniculate bodies, and the poorly-developed fiber network are indicative of a greatly reduced visual system. Due to this reduction in the visual system in the Insectivora, the olfactory sense may be considered to be a sensory system of compensation (Sigmund and Sedlacek, 1985; Stephan et al., 1991; Baron and Stephan, 1996).

The auditory sense is of greater importance to shrews than the visual system. As vocal mammals, shrews perceive and emit a wide range of sounds. The size of the external ear varies depending upon species and habitat, ranging from the most prominent ears in ground dwelling shrews to extremely minute structures. In semi-fossorial and semi-aquatic species of shrews, the external ear is completely lacking with only a narrow opening covered with a thin fold of skin (Churchfield, 1990). The importance of audition
to the shrew has not yet been demonstrated, but the brain stem and cortical regions involved with audition are not well-developed.

Additional sensory information is received via the trigeminal complex which relays information from the facial vibrissae to higher brain centers (Baron et al., 1990). The trigeminal complex size index varies significantly among the Insectivora (Baron et al., 1990). For example, in Blarina, the trigeminal complex has a size index of 140. In general, the terrestrial Insectivora have an average trigeminal complex size index of 134. In the Insectivora, the best-developed trigeminal complex with highly-developed vibrissae is found in the semi-aquatic forms. The average trigeminal complex size index in the semi-aquatic Insectivora is 283. Variations in the development of the trigeminal complex and vibrissae may account for olfactory acuity differences in the Insectivora.

1.3 The Importance of Olfaction

The term "macrosmatic" describes a species with a well-developed, well-organized olfactory system with high sensitivity for odorants. Macrosmatic mammals rely upon their olfactory system for various behaviors that is essential to their survival. The term "microsmatic" is typically used to describe a species with a poorly-developed olfactory system. The term "anosmatic" relates to the impairment or loss of the sense of smell. Primates are considered to be microsmatic mammals due to the size reduction in olfactory brain areas and reduced olfactory acuity and olfactory importance (Loo, 1977; Turner et al., 1978; Laska et al., 2000; Barbado et al., 2001). For the macrosmatic shrew, the olfactory system typically is highly-developed (Negus, 1958; Wohrmann-Repenning, 1975; Schmidt and Nadolski, 1979; Sollner and Kraft, 1980; Churchfield, 1990; Stephan et al., 1991) and is thought to be essential for numerous activities critical
for survival. These activities include reproductive and social behavior, recognition of prey and/or predators, aggressive behavior, detection of food, maternal-infant relationships, and habitat selection (Le Gros Clark, 1932; Allison, 1953b; Johnson, 1957; Negus, 1958; Wohrmann-Repennig, 1975; Schmidt and Nadolski, 1979; Hutterer, 1985; Larochelle and Baron, 1986, 1989a, b; Churchfield, 1990; Stephan et al., 1991; Farbman, 1992; Shipley et al., 1995; Shipley and Smith, 1995; Nieuwenhuys et al., 1998).

1.4 Statement of the Problem

Two Soricidae shrews, *Blarina brevicauda* and *B. carolinensis* were of particular interest for this research since both have very small eyes and cochleas and correspondingly small brain areas involved in visual and auditory sensation (Gould, 1969; Burda, 1979; Branis, 1981; Stephan et al., 1991). However, the olfactory system of shrews appears not only to be highly developed, but also a system that plays an important part of their sensory repertoire (Stephan et al., 1991). The present investigation was designed to provide a detailed ultrastructural analysis of the olfactory system of the two closely related soricid shrews in order to test the hypothesis that their olfactory systems are well-developed and differ significantly from those of better known small macrosmatic mammals. The olfactory system components investigated by light and electron microscopy for this research included the olfactory epithelium and mucosa, main and accessory olfactory bulbs, anterior olfactory nucleus, olfactory tubercle, and piriform cortex.

1.5 Olfactory Epithelium and Mucosa (OEM)

The olfactory mucosa consists of the epithelium, a basal lamina, and the underlying lamina propria. Within the lamina propria, Bowman’s glands, axon bundles, connective
tissue, and vasculature are common (Graziadei, 1971; Wohrmann-Repengning and Meinel, 1975; Morrison and Costanzo, 1989, 1992; Naguro and Iwashita, 1992; Mendoza, 1993). The olfactory epithelium is a pseudostratified epithelium consisting of receptor cells, supporting cells, and basal cells. The receptor cells and supporting cells are produced by mitosis and differentiation of basal cells throughout life (Graziadei and Graziadei, 1978, 1979; Barber and Raisman, 1979; Doucette et al., 1983; Morrison and Costanzo, 1989; Farbman and Buchholz, 1992; Oakley and Riddle, 1992; Hirai et al., 1996; Matsuoka et al., 2002). Receptor cell nuclei are typically located in the lower two-thirds of the epithelium. Supporting cells are columnar with microvilli on the apical surface with their nuclei located in the upper one-third of the epithelium (Morrison and Costanzo, 1989). Basal cells are elongated cells that lie adjacent to the basement membrane (Allison, 1953b; Arstila and Wersäll, 1967; Graziadei, 1971). Odor molecules stimulate first-order neurons, the olfactory bipolar receptor nerves in the olfactory epithelium within the nasal cavity. The axons of olfactory receptor nerves pass through the lamina propria and project via the olfactory nerve bundles to the main olfactory bulb which, in turn, projects to higher olfactory structures in the cerebral hemisphere (Graziadei, 1966, 1971, 1973; Anholt, 1987; Shipley et al., 1995).

Studies of the mammalian OEM by light and electron microscopy include Arstila and Wersäll (1967), Frisch (1967, 1969), Seifert (1970), Graziadei (1966, 1971, 1973), Adams (1972), Altner and Kolnberger (1975), Cuschieri and Bannister (1976), Yamamoto (1976), Dodd and Squirrel (1980), Breipohl and Ohyama (1981), and Menco (1980, 1983). However, few studies have examined the olfactory mucosa of members of the Order Insectivora and only four of these were ultrastructural studies including

1.6 Main Olfactory Bulb (MOB)

The MOB has been reported to be a phylogenetically old component of the cerebral cortex (Kosaka and Kosaka, 1999; Gil-Carcedo et al., 2000). The MOB is attached to the rostral end of the telencephalon by the olfactory peduncle. As a rostral expansion of the forebrain, the MOB is the site of the first synaptic interactions of the epithelial receptor cells, contains the second order neurons of the olfactory pathway, and functions as a relay station for olfactory signals between the olfactory mucosa and higher olfactory centers. Primary projections from the MOB terminate in the anterior olfactory nucleus, piriform cortex, and the olfactory tubercle. Projections to the MOB originate in the anterior olfactory nucleus, piriform cortex, nucleus of the lateral olfactory tract, and the hypothalamus (Smith, 1896; Young, 1934; Davis et al., 1978; Baron et al., 1983; Carson, 1984; Greer, 1991; Bassett et al., 1992; Eisthen, 1997; Nieuwenhuys et al., 1998; Gil-Carcedo et al., 2000). The MOB consists of six concentric layers, starting with the outermost include the olfactory nerve layer, glomerular layer, external plexiform layer, mitral cell layer, internal plexiform layer, and granule cell layer. There is a general decrease in size of the MOB from the Insectivora to higher primates with man having a very small MOB. In some mammals, such as cetaceans, the MOB is absent. In general, a positive relationship exists between MOB size and the importance of olfaction to a species (Johnston, 1909; Allison, 1953b; Baron et al., 1983; Farbman, 1992; Shipley et al., 1995).

The majority of MOB research has focused on light/immunohistochemical microscopic studies (Forbes, 1984; Mouradian and Scott, 1988; Sanides-Kohlrusch and
Wahle, 1990; Bassett et al., 1992; Crespo et al., 1995; Kasowski et al., 1999; Treloar et al., 1999; Brinon et al., 2001; Huang and Bittman, 2002). Relatively few studies have examined the ultrastructure of the MOB (Klinkerfuss, 1964; Andres, 1970; Price and Powell, 1970c; Switzer and Johnson, 1977; Jackowski et al., 1978; Gomez and Potts, 1981; Hinds and McNelly, 1981; Sturrock, 1981; Doucette, 1984; Lopez-Mascaraque et al., 1986; Moriiizumi et al., 1995; Chuah et al., 1997). Little research has been done on the MOB of the Insectivora.

1.7 Accessory Olfactory Bulb (AOB)

The main olfactory system and the vomeronasal olfactory system (accessory olfactory system) are reported to be separate pathways from their point of origin in the nasal mucosa to their termination site. Therefore, it has been hypothesized that two separate olfactory systems exist (Scalia and Winans, 1975; Davis et al., 1978; Takami and Graziadei, 1990; Shipley and Smith, 1995). The main olfactory system plays a major role in food location and prey detection whereas the vomeronasal olfactory system has been proposed to function in mammals in reproductive behaviors, mediating pheromonal communication, pregnancy, puberty, ovulation, and aggression (Coquelin et al., 1984; Imamura et al., 1985; Wysocki and Meredith, 1991; Takami et al., 1992; Doving and Trotier, 1998; Jansen et al., 1998; Dudley and Moss, 1999; Wekesa and Anholt, 1999; Johnston and Peng, 2000; Brennan, 2001; Korschling, 2001; Sahara et al., 2001; Kondo et al., 2003).

The projection pathways for the AOB are significantly more simple than the pathways for the MOB. Axons of the AOB mitral/tufted cells form the accessory olfactory tract which runs with the lateral olfactory tract and projects to the amygdala.
Other AOB projection sites within the cortex include the bed nucleus of the accessory olfactory tract, the bed nucleus of the stria terminalis, the supraoptic nucleus, and the ventromedial hypothalamic nucleus (Scalia and Winans, 1975, 1976; Barber et al., 1978; Barber and Raisman, 1978; De Olmos et al., 1978; Johns, 1980; Lehman and Winans, 1982; Segovia et al., 1982; Raisman, 1985; Saito and Moltz, 1986; Brennan et al., 1990; Price, 1991; Wysocki and Meredith, 1991; Shipley et al., 1995; Matsuoka et al., 1997; Meisami and Bhatnagar, 1998; Dudley and Moss, 1999; Jia et al., 1999; Keverne, 1999; Sugai et al., 1999; Goldmakher and Moss, 2000; Breer, 2001; Salazar and Brennan, 2001; Cloutier et al., 2002; Kondo et al., 2003). Centrifugal fibers that originate in the amygdala project to the AOB (Shipley et al., 1995; Meisami and Bhatnagar, 1998). The AOB is laminated similar to the MOB but the layers are less distinct and less developed.

Few light and electron microscopy studies have focused on the vomeronasal organ (Kratzing, 1971; Barber and Raisman, 1979; Breipohl et al., 1981; Vaccarezza et al., 1981; Fraher, 1982; Adams and Wiekamp, 1984; Mendoza, 1986; Mendoza and Kuhnel, 1987; Matsuzaki et al., 1993; Doving and Trotier, 1998; Poran, 1998; Smith et al., 1998; Zuri et al., 1998). Several histochemical and immunohistochemical studies have focused on the AOB (Carson and Burd, 1980; Salazar et al., 1998; Salazar and Quinteiro, 1998; Dudley and Moss, 1999; Nakamura et al., 1999; Wekesa and Anholt, 1999; Takigami et al., 2000; Sahara et al., 2001; Salazar and Brennan, 2001; Salazar et al., 2001; Kondo et al., 2003) whereas very few ultrastructural studies have examined the AOB (Matsuoka et al., 1997, 1998).
1.8 Anterior Olfactory Nucleus (AON)

The olfactory peduncle links the MOB to the forebrain and consists of two components, the anterior olfactory nucleus (AON) and various transition areas between the AON and the olfactory cortex (Bayer, 1986; Garcia-Ojeda et al., 1998). Herrick (1924) first introduced the term “nucleus olfactorius anterior” to describe the gray matter just caudal to the MOB. According to Herrick, the organization of the AON shows significant variation in different mammal species. Due to the heavy reciprocal connections between the AON and the MOB, the AON has been proposed to function as a relay for interhemispheric communication between the two MOBs via the anterior limb of the anterior commissure (Bennett, 1968; Broadwell, 1975b; Brunjes and Frazier, 1986; Bassett et al., 1992; Haberly, 2001). Most researchers now agree that the AON is actually a cortical structure (Shipley et al., 1995). The divisions of the AON are laminated and consist of an outer plexiform layer and a homogeneous inner layer of AON pyramidal cells (Davis and Macrides, 1981b; Shipley and Ennis, 1996). This bilayer form of cortex has been recognized in all mammals examined, including the opossum (Shammah-Lagnado and Negrao, 1981), hedgehog (Valverde et al., 1989), rabbit (Broadwell, 1975b), rat (Haberly and Price, 1978b, Bayer, 1986; Garcia-Ojeda et al., 1998), mouse (Crosby and Humphrey, 1939a), and cat (Ryu, 1980). The mammalian AON generally is subdivided into six subdivisions including the pars lateralis, ventralis, medialis, dorsalis, posterior, and externa (Davis and Macrides, 1981b).

1.9 Olfactory Tubercle (OT)

The OT is a trilaminar cortical structure consisting of plexiform, pyramidal, and polymorph cell layers (Talbot et al., 1988a; Krieger, 1981; Krieger and Scott, 1989). The
general size of the OT is greatly influenced by the size of other olfactory structures, such as the MOB (Stephan et al., 1991). In macrosmatic mammals, the OT is well-developed (Johnson, 1957a; Millhouse and Heimer, 1984; Berezhnaya et al., 1999) whereas in microsmatic mammals such as humans (Crosby and Humphrey, 1941), the OT is poorly-developed. The OT receives projections from the MOB via the lateral olfactory tract (Davis and Macrides, 1981; Meyer, 1981; Krieger, 1981; Fallon, 1983; Bassett et al., 1992). The OT differs from the AON and the piriform cortex because the OT does not send reciprocal projections back to the MOB (Shipley et al., 1995). The OT also receives projections from other regions of the cortex including the PC, AON, and structures within the limbic system including the hippocampus and amygdala. The OT sends projections to the globus pallidus, substantia nigra, and thalamus. Due to the extensive projections, it has been proposed that the OT is not only a part of the olfactory system, but also is a part of the limbic system (Herrick, 1921; Fallon et al., 1978; Haberly and Price, 1978b; Davis and Macrides, 1981a; Krieger, 1981; Meyer 1981; Guevara-Aguilar et al., 1982; Luskin and Price, 1983b; Meyer and Wahle, 1986; Stephan et al., 1991; Kratskin, 1995).

The majority of OT research has focused on histochemical analysis of enzyme concentrations (Okada et al., 1977; Gilad and Reis, 1979; Gordon and Krieger, 1983; Talbot et al., 1988b; Krieger and Scott, 1989) or various neurotransmitters (Krieger et al., 1983). Several studies have examined the morphology of OT by light microscopy (Fallon et al., 1978; Guevara-Aguilar et al., 1982; Ribak and Fallon, 1982; Fallon, 1983; Gordon and Krieger, 1983; Krieger et al., 1983; Talbot et al., 1988a; Krieger and Scott, 1989; Berezhnaya et al., 1999) and have focused typically on Golgi-stained sections and have concluded that the OT of macrosmatic mammals is typically large and well-
developed. Few studies have examined the OT by transmission electron microscopy (Krieger, 1981; Ribak and Fallon, 1982; Krieger et al., 1983; Zahm and Heimer, 1985; Krieger and Scott, 1989; Josephson et al., 1997; Berezhnaya et al., 1999).

1.10 Piriform Cortex (PC)

The piriform cortex is also called the pyriform or prepyriform cortex and is a phylogenetically old structure of the paleocortex (Haberly, 1985). The PC is located on the ventrolateral surface of the brain and is divided into three layers including the outermost superficial Layer I, middle Layer II, and deep Layer III. Layer I contains axons and terminals of MOB mitral/tufted cells and dendrites of Layer II and III pyramidal cells. Layer II is a cell layer of tightly packed pyramidal cells that send apical dendrites toward Layer I. Layer III contains pyramidal cells and multipolar cells. Dendrites of Layer III multipolar neurons extend in all directions throughout Layer III whereas dendrites of Layer III pyramidal cells extend toward Layer I (Davis and Macrides, 1981b; Shipley and Ennis, 1996; Bartolomei and Greer, 1998; Löscher et al., 1998). The PC pyramidal cells of Layers II and III project to the ipsilateral MOB (Davis and Macrides, 1981b; Shipley et al., 1995) and provide excitatory input to MOB granule cells (Stripling and Patneau, 1999; Fukushima et al., 2002). The PC also receives projections from the ipsilateral MOB (David and Macrides, 1981; Bassett et al., 1992; Shipley et al., 1995; Rosin et al., 1999).

Few studies have focused on the PC and include Haberly and Lewis (1978a, b); Davis and Macrides (1981); Meyer (1981); Luskin and Price (1983b); Friedman and Price (1984); Ojima et al., (1984); Curcio et al., 1985; Haberly, (1985); Anders and Johnson (1990); Bartolomei and Greer, 1998; Löscher et al., (1998); Rosin et al., (1999);
Johnson et al., 2000; Protopapas and Bower (2000); Datiche et al., (2001); Haberly (2001); Fukushima et al., (2002); Best and Wilson (2003). Investigation of the Insectivora PC (Ryzen and Campbell, 1955; Stephan and Andy, 1982) has been significantly neglected.
CHAPTER II

METHODS

2.1 Introduction

Fifteen short-tailed shrews, 9 *Blarina carolinensis* and 6 *B. brevicauda*, were captured in the vicinity of Norfolk, Virginia for these ultrastructural studies. Mature shrews of both sexes were used. Handling of animals was carried out in accordance with our animal care and use committee guidelines, which are based on National Institutes of Health guidelines found in *Principles of Animal Care* (NIH publication No. 86-23).

2.2 Light Microscopy of Epoxy-Embedded Tissues

For the ultrastructural studies, shrews were lightly etherized and then injected intraperitoneally with the primary anesthetic, 0.2 ml of 2.5% tribromoethanol. After waiting about 2 minutes for the shrews to become deeply anesthetized, the chest was opened and the vascular system was perfused via a 22-gauge needle through the left ventricle to flush out the blood and deliver the fixative to all body tissues quickly. The perfusion bottle was suspended approximately 3 feet above the shrews. The right atrium was cut with small scissors to provide an outlet for the perfusate. The animals were perfused for about 2 mins with about 10 ml solution of 0.9% sodium chloride, 1% sodium nitrite, 5 mg/100 ml heparin, and 0.05 M phosphate buffer at pH 7.4, followed by the primary fixative consisting of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer pH 7.4 for about 30 mins. During the perfusion of fixative, a syringe was used to deliver additional fixative into each nasal passage. The OEM pieces were dissected and left overnight in primary fixative at 4° C. For the MOB, AOB, AON, OT,
and PC, brains were cut on a vibratome at 200 μm and the specific areas cut from slices. The tissues were then rinsed in cold 0.1 M cacodylate buffer pH 7.4 three times, 10 mins each. The tissues were then post-fixed for 2 hrs at 4° C in 2% osmium tetroxide in 0.1 M cacodylate buffer pH 7.4 and then rinsed with cold 0.1 M cacodylate buffer, three times, 10 mins each. The tissues were dehydrated in 10-minute steps with 30%, 50%, 70%, 95%, and 100% (two times) ethanol followed by 100 % acetone (two times). The tissues were infiltrated with a 1:1 mixture of epoxy resin (Polybed 812, Polysciences) and acetone for 4 hrs on a rotating mixer followed by 18 hrs in 100% epoxy resin. The OEM and MOB were embedded in flat molds and polymerized for 48 hrs at 65° C. The AOB, AON, OT, and PC were embedded in epoxy resin between two Teflon-coated coverslips and polymerized for 48 hrs at 65° C. These tissues were cut from the coverslip wafer using a scalpel and were glued onto epoxy blocks. One-micrometer sections of each type of olfactory system tissue were cut for light microscopy and stained with methylene blue/azure II stain of Richardson and coworkers (1960). Four slides of each species were used to count dark and light supporting cells and Bowman’s gland cells. In each slide, sample areas visible at 400X were randomly selected and cells were counted to arrive at an estimation of the relative percentages. Identification of light microscopy structures of the olfactory tubercle and piriform cortex were based upon Millhouse and Heimer (1984) and Haberly and Feig (1983) respectively.

2.3 Transmission Electron Microscopy of Epoxy-Embedded Tissues

Silver thin sections of all olfactory system tissues were cut with a diamond knife on a RMC MT2C ultramicrotome (Research and Manufacturing Co., Tucson, AZ) and placed on naked copper grids. The sections were stained with uranyl acetate for 20 mins and
lead citrate for 3 mins. The tissues were viewed and photographed using a JEOL 100CX II transmission electron microscope (TEM). Identification of the fine structure of the olfactory mucosa was based upon the work of Frisch (1967) and Yamamoto (1976). Identification of neurons and processes of the main olfactory bulb was based upon the work of Pinching and Powell (1971a, b, c). Identification of neurons and processes of the accessory olfactory bulb was based upon the work of Barber and coworkers (1978). Structures of the anterior olfactory nucleus were based upon the work of Valverde and coworkers (1989) and Crosby and Humphrey (1941).

2.4 Scanning Electron Microscopy of Olfactory Epithelium Tissues
The olfactory epithelium selected for SEM was fixed and dehydrated as previously described. Following dehydration these samples were critical point dried using liquid carbon dioxide, mounted on aluminum stubs and then coated with a thin layer of gold/palladium alloy. The samples were then viewed and photographed in a LEO 435VP SEM.

2.5 Light Microscopic Histochemistry of Frozen Sections
Shrews used for cresyl violet light microscopy studies were anesthetized as described previously and then perfused with 4% depolymerized paraformaldehyde in 0.1M phosphate buffer, pH 7.4. The heads were removed, skinned and fixed for an additional 48 hrs at 4°C. The brain was removed and soaked in the cryoprotectant, 30% sucrose buffer for 24 hours to prevent ice crystal formation. The brain was placed onto the sliding/freezing microtome chuck, frozen with dry ice, and sectioned at 40 μm. Sections were picked up from the microtome blade with a brush and placed in a tray containing
phosphate buffer rinse. Sections were then mounted on gelatin-coated slides and dried overnight. The tissue was treated for 4-8 hours with chloroform, then 100% acetone. Following acetone, the sections were rinsed in 95% ethanol then 70% ethanol. Sections were stained with 1% cresyl violet for 20 minutes. The sections were washed for several minutes in water. The tissue was dehydrated through 70%, 95% and 100% ethanol followed by chloroform for 5 mins. A differentiator of 95% ethanol with glacial acetic acid, pH 4.1 was used for 5-7 mins to remove any remaining cresyl violet stain. The sections were then dehydrated with 95% and 100% ethanol followed by xylene and coverslipped with Permount.

2.6 Light Microscopic Histochemistry of Paraffin-Embedded Tissues

Shrews used for hematoxylin and eosin light microscopy were anesthetized as described previously and then perfused with 4% depolymerized paraformaldehyde. The heads were removed, skinned and fixed for an additional 48 hrs at 4°C. The heads were then decalcified in a solution of formic acid (25%) and sodium citrate (10%) for 24 hours at room temperature and then rinsed in running water for 4 hours. The heads were then dehydrated and embedded in paraffin using standard techniques. The heads were sectioned in the coronal plane at 10 micrometers and the sections were picked up on slides. Alternate slides were stained for routine histological study with hematoxylin and eosin, and for mucopolysaccharides with the periodic acid Schiff-alcian blue stain (Preece, 1972).

2.7 Table of Abbreviations

Table 1 is a list of abbreviations used in the text of this research project.
Table 1. Abbreviations of olfactory system structures.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOB</td>
<td>accessory olfactory bulb</td>
</tr>
<tr>
<td>AON</td>
<td>anterior olfactory nucleus</td>
</tr>
<tr>
<td>EPL</td>
<td>external plexiform layer</td>
</tr>
<tr>
<td>EP/M-TCL</td>
<td>external plexiform layer/mitral-tufted cell layer</td>
</tr>
<tr>
<td>ETC</td>
<td>external tufted cell</td>
</tr>
<tr>
<td>GL</td>
<td>glomerular layer</td>
</tr>
<tr>
<td>GrL</td>
<td>granule cell layer</td>
</tr>
<tr>
<td>IPL</td>
<td>internal plexiform layer</td>
</tr>
<tr>
<td>MCL</td>
<td>mitral cell layer</td>
</tr>
<tr>
<td>MOB</td>
<td>main olfactory bulb</td>
</tr>
<tr>
<td>OEM</td>
<td>olfactory epithelium and mucosa</td>
</tr>
<tr>
<td>ONL</td>
<td>olfactory nerve layer</td>
</tr>
<tr>
<td>OT</td>
<td>olfactory tubercle</td>
</tr>
<tr>
<td>PC</td>
<td>piriform cortex</td>
</tr>
<tr>
<td>PD</td>
<td>pars dorsalis of the anterior olfactory nucleus</td>
</tr>
<tr>
<td>PE</td>
<td>pars externa of the anterior olfactory nucleus</td>
</tr>
<tr>
<td>PGC</td>
<td>periglomerular cell</td>
</tr>
<tr>
<td>PL</td>
<td>pars lateralis of the anterior olfactory nucleus</td>
</tr>
<tr>
<td>PM</td>
<td>pars medialis of the anterior olfactory nucleus</td>
</tr>
<tr>
<td>PP</td>
<td>pars posterior of the anterior olfactory nucleus</td>
</tr>
<tr>
<td>PV</td>
<td>pars ventralis of the anterior olfactory nucleus</td>
</tr>
<tr>
<td>RER</td>
<td>rough endoplasmic reticulum</td>
</tr>
<tr>
<td>SAC</td>
<td>short-axon cell</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscopy</td>
</tr>
<tr>
<td>SER</td>
<td>smooth endoplasmic reticulum</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
</tr>
<tr>
<td>VNL</td>
<td>vomeronasal nerve layer</td>
</tr>
<tr>
<td>VNO</td>
<td>vomeronasal organ</td>
</tr>
</tbody>
</table>
CHAPTER III

OLFACTORY EPITHELIUM AND MUCOSA

3.1 Introduction

In mammals, the olfactory mucosa consists of the olfactory epithelium and the underlying lamina propria. The olfactory epithelium is a pseudostratified epithelium that contains bipolar receptor cells, supporting cells, and basal cells. The lamina propria contains vasculature, Bowman’s glands, and axon bundles (Morrison and Costanzo, 1992; Naguro and Iwashita, 1992; Mendoza, 1993). The olfactory epithelium and mucosa have been studied in numerous macrosmatic mammal species including a mole (Graziadei, 1966), shrew (Carson et al., 1994), hedgehog (Erhardt and Meinel, 1979), bat (Yamamoto, 1976), guinea pig (Arstila and Wersäll, 1967), rabbit (DeLorenzo, 1957; Zinnin, 1964; Yamamoto, 1976), mouse (Frisch, 1967, 1969; Cuschieri and Bannister, 1975), hamster (Morrison and Costanzo, 1989), rat (Hinds and McNelly, 1981; Carr et al., 1991; Naguro and Iwashita, 1992; Weiler and Farbman, 1997), dog (Okano et al., 1967), and cat (Graziadei, 1964). The olfactory epithelium and mucosa have also been studied in only a few microsmatic mammals including the rhesus monkey (Shantha and Nakajima, 1970) and primates including the loris, macaque, and gibbon (Loo, 1977), and humans (Moran et al., 1982a, b; Morrison and Costanzo, 1990, 1992).

3.2 Results

3.2.1 Light Microscopy Overview

The olfactory mucosa (Fig. 1) varied in thickness from 35 to over 150 μm and consisted of the overlying olfactory epithelium, a thin basal lamina, and a lamina propria that

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
contained Bowman's glands, bundles of axons, vasculature and connective tissue. The olfactory epithelium was a pseudostratified columnar epithelium that contained five to ten layers of cell nuclei. Five cell types (Fig. 2) were common in the olfactory epithelium including receptor cells, two types of supporting cells, and two types of basal cells.

3.2.2 Light Microscopy of Receptor Cells

The receptor cell nuclei (Fig. 2) were located within the lower two-thirds of the olfactory epithelium. The oval, darkly-stained nuclei were more irregular in shape than the other epithelial nuclei and typically had a distinct nucleolus. Small patches of heterochromatin were scattered throughout the receptor cell nucleus. The receptor cell nuclei measured up to 6 μm in diameter. Receptor cells had a single apical dendrite that was topped by a bulbous olfactory knob that projected above the apical surfaces of the supporting cells (Fig. 2).

3.2.3 Light Microscopy of Supporting Cells

Supporting cell nuclei were located within the upper one-third of the olfactory epithelium. Two populations of supporting cells, including dark supporting cells and light supporting cells were observed. The dark supporting cells (Fig. 2) had an oval nucleus with scattered patches of heterochromatin. These cells were columnar in shape with numerous long microvilli on the apical surface. The nuclei measured up to 6 μm in diameter. The average number of dark supporting cells per random sample area was approximately 10. The light supporting cell cytoplasm and nucleus were much more lightly stained than that of the adjacent dark supporting cells. The light supporting cell nuclei (Fig. 2) measured up to 6 μm in diameter. These light supporting cells averaged...
Fig. 1. Light micrograph of the olfactory epithelium (EPI). Lamina propria consists of Bowman's glands (BG), axon bundles (AX) and vasculature (V). Basal lamina (arrow). 1 μm epoxy resin section, Richardson stain. 5 mm = 4 μm.

Fig. 2. Light micrograph of the five cell types of the olfactory epithelium. Receptor cells (R) topped by a dendritic knob (arrowhead) that extends above the apical surface of the supporting cells, dark supporting cells (DS), light supporting cells (LS), dark basal cells (B), and light globose basal cells (arrow). 1 μm epoxy resin section, Richardson stain. 5 mm = 4 μm.
only 1 cell per sample area and were less numerous than the dark supporting cells, making up just 8-10% of the supporting cells.

3.2.4 Light Microscopy of Basal Cells

Basal cells were typically flattened and were located in the lower one-third of the olfactory epithelium just above the basal lamina (Fig. 2). The dark basal cell had darkly-stained nuclei and contained small patches of heterochromatin. Dark basal cell nuclei measured up to 3 μm in diameter along the short axis and 6 μm in diameter along the long axis. Light globose basal cells (Fig. 2) had euchromatic nuclei with small amounts of heterochromatin. The light basal cell nuclei measured up to 4 μm in diameter along the short axis and 5 μm in diameter along the long axis.

3.2.5 Light Microscopy of Lamina Propria Axon Bundles

The structures of the lamina propria included olfactory nerve axon bundles with ensheathing cells, Bowman’s glands, vasculature, and connective tissue. Olfactory nerve axon bundles (Fig. 3) were oval or spherical in cross-section and measured up to 30 μm by 60 μm. These bundles were especially large and numerous in areas where the olfactory epithelium was thickest. Ensheathing cells (Fig. 3) were often located at the perimeter of axon bundles and had nuclei that measured up to 4 μm in diameter along the short axis and 5 μm in diameter along the long axis. These cells typically had a euchromatic nucleus. Occasionally a distinct nucleolus was evident. Some ensheathing cells had light nuclei and some had darker nuclei. Large axon bundles were often separated into fascicles by thin septa made up of ensheathing cell processes.
3.2.6 Light Microscopy of Lamina Propria Bowman's Glands

Two types of Bowman’s gland cells could be distinguished in the lamina propria. The more common of these, the mucous cell (Fig. 3), had moderately basophilic cytoplasm that often contained pale granules. These cells were grouped in an acinar arrangement around a small lumen. The pale granules were usually more numerous adjacent to the lumen. Another moderately basophilic gland cell, the serous cell (Fig. 3), was often observed in the same acinus with the mucous cells. These cells contained small, darkly-stained secretory granules that were also aggregated near the lumen. Per sample area, mucous Bowman’s gland cells outnumbered serous cells in a ratio of about five to one. The nuclei of the Bowman’s gland cells were oval in shape and measured up to 6 μm in diameter. In PAS-alcian blue stained sections, there was a distinctive staining of the Bowman’s gland cells. The mucous cells were alcian blue-positive and the serous cells were PAS-positive.

3.2.7 Light Microscopy of Lamina Propria Vasculature

The lamina propria was highly vascularized by capillaries (Fig. 3) that measured up to 24 μm in diameter. In the spaces between the vessels, Bowman’s glands, and the olfactory nerve axon bundles, there was a small amount of connective tissue, mostly consisting of fibroblasts and collagen fibrils.

3.2.8 Transmission Electron Microscopy of Receptor Cells

Receptor cell (Fig. 4) nuclei were spherical with some electron dense patches of heterochromatin. The perikarya contained numerous mitochondria densely packed in the apical cytoplasm, lysosomes, and multivesicular bodies. Dispersed strands of rough
Fig. 3. Light micrograph of the axon bundle (AX) of the lamina propria. Pale staining ensheathing cells (arrow), vasculature (V), mucous cells (M) and serous cells (curved arrow) of Bowman’s glands. Bowman’s gland duct (arrowhead) penetrates the basal lamina and passes up through the epithelium to the surface. 1 µm epoxy resin section, Richardson stain. 5 mm = 5 µm.

Fig. 4. TEM micrograph of the receptor cell (R) of the olfactory epithelium. A well-developed Golgi (arrow) is located in the basal cytoplasm. Cilia (arrowhead) extend from the dendritic knob. 5 mm = 1 µm.
endoplasmic reticulum (RER) and a well-developed Golgi were located in the apical and basal cytoplasm. A single dendrite extended to the epithelial surface where it formed a bulbous knob from which several cilia extended. The dendritic knobs (Fig. 5) measured up to 3 μm high by 2 μm wide. Ciliary rootlets were often found in dendrites. Longitudinal sections of cilia showed significant tapering a short distance from the dendritic knob (Fig. 6). Numerous spherical membranous bodies 0.6-1.1 μm in diameter were observed among the cilia. In some instances, a cilium was observed to be directly connected to a spherical body (Fig. 6). Some cilia also appeared to have dilations along their length. A dense carpet of cilia was observed on the epithelial surface by scanning electron microscopy (Fig. 7). Cilia were 200-260 nanometers in diameter at their base and contained the typical 9 + 2 axonemal arrangement of microtubules (Fig. 8). In distal parts of the cilia, only two microtubules were present.

3.2.9 Transmission Electron Microscopy of Supporting Cells

The dark supporting cell (Fig. 9) had moderately dense cytoplasm and nucleus. The apical surface of the dark supporting cell had numerous long microvilli up to 2 μm in length, some of which were branched and irregular in shape. The plasma membrane at the base of the microvilli often had subsurface cisternae. The apical cytoplasm contained high amounts of smooth endoplasmic reticulum (SER). In many dark supporting cells, SER cisternae showed an unusual parallel arrangement. Golgi apparatus was commonly located in the apical cytoplasm. Light supporting cells (Fig. 9) had a convex apical surface with fewer microvilli than the dark supporting cells. The light supporting cell apical cytoplasm contained very small amounts of SER and large numbers of mitochondria. The light supporting cell nucleus was oval and more electron-lucent than
Fig. 5. TEM micrograph of the receptor cell dendrite. Mitochondria (M) fill the receptor cell apical dendrite. The dendritic knob (arrowhead) contains numerous basal bodies of cilia. Extensive, irregularly shaped microvilli (curved arrow) extend from the apical surface of the dark supporting cells (DS). 5 mm = 0.4 μm.

Fig. 6. TEM micrograph of the cilium of the receptor cell. Tapering of the cilium (arrow), swelling at the tip of cilium (arrowhead). 5 mm = 0.6 μm.
Fig. 7. SEM micrograph of the dense carpet of cilia. Numerous spherical swellings along the cilia (arrow). 5 mm = 1.0 μm.

Fig. 8. TEM micrograph of the microtubule arrangement. Microvillus (arrow), cross-section of a receptor cell cilium (arrowhead) showing the 9 + 2 axonemal arrangement of microtubules. 5 mm = 0.05 μm.
the dark supporting cell nucleus.

3.2.10 Transmission Electron Microscopy of Basal Cells

Basal cells (Fig. 10) were typically adjacent to the basal lamina. The basal cell cytoplasm contained numerous polyribosomes and relatively little endoplasmic reticulum. Dark basal cell nuclei were heterochromatic and more electron-dense than the light globose basal cell nuclei (Fig. 10). Occasionally, basal cell processes incompletely surrounded small groups of receptor cell axons. Axons typically were not separated from each other by any cell processes in the bundles. The basal lamina underlying the epithelium was not especially prominent or thickened.

3.2.11 Transmission Electron Microscopy of Lamina Propria Axon Bundles

Large bundles of small unmyelinated axons were common throughout areas of olfactory mucosa (Fig. 11). The axons were 0.2-0.4 μm in diameter. Each bundle of axons was surrounded by a layer of ensheathing cell processes. In some of the larger axon bundles, ensheathing cell processes formed septa between groups of axons. The membranes of adjacent axons were not separated by any visible cellular structures or extracellular matrix. Ensheathing cells in the lamina propria were surrounded by a thin basal lamina.

3.2.12 Transmission Electron Microscopy of Lamina Propria Bowman’s Glands

Two types of Bowman’s gland cells (Fig. 12) were distinguished. The most common, the mucous cell, contained electron-lucent secretory granules with fine-grained contents. The cytoplasm of these cells contained large amounts of SER and small aggregates of RER. Secretory granules were most numerous in the apical cytoplasm adjacent to the acinar lumen. The second cell type, the serous cell, contained large areas of RER, and a
Fig. 9. TEM micrograph of two types of supporting cells. Dark supporting cells (DS) with numerous, irregular microvilli (arrowhead). The apical cytoplasm contains large amounts of smooth endoplasmic reticulum (SER). Light supporting cells (LS) have a convex apical surface with microvilli (arrow). 5 mm = 1 µm.

Fig. 10. TEM micrograph of two types of basal cells. Dark basal cells (DB) rest upon the basal lamina (arrow). Basal cell processes and perikarya incompletely surround groups of receptor cell axons (A). Light globose basal cell nuclei (LB). 5 mm = 0.7 µm.
Fig. 11. TEM micrograph of the axon bundle. The ensheathing cell nucleus (ES) lies at the perimeter of the bundle of unmyelinated axons (A). 5 mm = 1 μm.

Fig. 12. TEM micrograph of the Bowman’s glands. Mucous cells (M) and serous cells (S) are organized in an acinar arrangement surrounding a lumen (L). 5 mm = 1 μm.
small amount of SER. Most of the serous granules were smaller than the mucous granules and were significantly more electron-dense. Secretory granules were not observed in the cytoplasm of the Bowman’s gland duct cells that passed through the epithelium to the surface of the mucosa.

3.3 Discussion

The basic organization of the olfactory epithelium and mucosa of *Blarina* is well-developed and is similar to other macrosmatic mammals (Allison, 1953a; Andres, 1966; Graziadei, 1966; Arstila and Wersäll, 1967; Frisch, 1967, 1969; Steinbrecht, 1969; Seifert, 1970; Cuschieri and Bannister, 1975; Yamamoto, 1976; Loo, 1977; Kratzing, 1978; Moran et al., 1982a, b; Morrison and Costanzo, 1992; Naguro and Iwashita, 1992). However, several differences were observed in *Blarina*.

3.3.1 Receptor Cells

The receptor cells in *Blarina* were comparable to receptor cells in other mammals. These cells are bipolar neurons with a dendrite at the apical pole and an axon extending from the basal pole. The dendrite forms a spherical-shaped swelling known as the olfactory knob, dendritic bulb, or olfactory vesicle. Numerous cilia extend from this knob (Naguro and Iwashita, 1992; Mendoza, 1993). Approximately 12-18 cilia extended from the dendritic knob on each receptor cell in *Blarina*. Two members of the order Insectivora, the mole, *Talpa europaea*, is reported to have only 1-5 olfactory cilia per receptor (Graziadei, 1966) and the hedgehog, *Erinaceus europaeus*, has about 12 cilia per receptor cell (Erhardt and Meinel, 1979). Mendoza (1993) reported approximately 11 cilia per dendritic knob in the rat. In the blind mole rat, *Spalax ehrenbergi*, (Zuri et al., 1998), an
average of 59 cilia per dendritic knob was reported. Cilia in *Blarina* exhibit the typical 9 + 2 microtubule pattern and intermediary vesicles and vesiculated tips, which have been noted in other species (Frisch, 1969; Graziadei, 1971; Menco, 1983; Burton, 1992). The mole olfactory receptors lack ciliary rootlets (Graziadei, 1966). However, rootlets were observed in *Blarina*. The receptor cell diameter in *Blarina* measured up to 6 μm which is comparable to other reports (Graziadei, 1971). The receptor cell axon in *Blarina* measured up to 0.4 μm in diameter whereas, Altner and Kohnberger (1975) report axon diameters to be no larger than 0.2 μm in diameter. Mendoza (1993) reports axon diameter in the rat to measure up to only 0.2 μm in diameter.

### 3.3.2 Supporting Cells

Supporting cells are columnar in shape with microvilli extending from the apical surface (Morrison and Costanzo, 1989; Naguro and Iwashita, 1992). The dark supporting cell of *Blarina* was very similar to the most common type of supporting cell reported in other mammals (Okano et al., 1967; Graziadei, 1971, 1973; Yamamoto, 1976). However, the light supporting cell of *Blarina* had features not reported for supporting cells in any other mammal. The apical surface of the light supporting cell of the shrew is similar to the apical surface of the microvillar cells that have been reported in the dog (Okano et al., 1967) and human (Moran et al., 1982a, b), although the microvillar cell has been reported to have an axon and may have a sensory function. The basal process of the light supporting cell of *Blarina* did not appear to be an axon since it is quite wide, contains rough endoplasmic reticulum, and lacks the microtubules characteristic of axons. The light supporting cell of *Blarina* has some similarity to the IA-6 reactive supporting cells described in the rat olfactory epithelium (Carr et al., 1991), especially with respect to the
protruding apical surface covered with microvilli. The IA-6 reactive cell is reported to be non-neuronal and appears to also lack the large amounts of SER found in most olfactory epithelium supporting cells. Meinel and Erhardt (1978) reported the presence of apical protuberances on supporting cells in the mole olfactory epithelium. These were not observed in Blarina. Graziadei (1966) did not report supporting cell apical protuberances in the mole olfactory epithelium. The function of the light supporting cell in the shrew is not known. However, the presence of many mitochondria and large numbers of small vesicles supports the hypothesis that this cell could be involved in transporting molecules or ions between the lamina propria and the fluid layer surrounding the olfactory receptor cilia.

The microvilli of these supporting cells in Blarina measured up to 2 μm in length which is shorter than the microvilli length in the cat which measured up to 5 μm in length (Graziadei, 1971). According to Graziadei, (1971) no relationship can be established regarding the number and length of microvilli in various vertebrates.

3.3.3 Basal Cells

Light and dark basal cells are reported to lie near the basal lamina between the basal processes of supporting cells and contain little cytoplasm around the nucleus (Arstila and Wersäll, 1967; Graziadei, 1971; Naguro and Iwashita, 1992; Mendoza, 1993). In the mouse (Frisch, 1967) and rat (Mendoza, 1993) basal cells have few distinguishable features. The light and dark basal cells in Blarina had few distinguishing features and were comparable to those of other mammal species.
3.3.4 Lamina Propria

Bowman’s glands are reported to be alveolar glands with ducts that open at the epithelial surface (Graziadei, 1971; Mendoza, 1993). The Bowman’s glands in the lamina propria of Blarina olfactory mucosa contained two cell types. The cell with the electron-lucent granules is similar to mucous cells observed in mammalian salivary glands whereas the cell type with dense granules is similar to salivary gland cells often classified as serous (Pinkstaff, 1980). Seifert (1970) reported that Bowman’s glands of several mammalian species had light and dark secretory cells, each containing secretory granules with different morphology, one with electron-dense granules and the other with pale granules. Similar secretory granules have been reported in the mouse (Frisch, 1967). Human Bowman’s glands are reported to contain only serous cells with electron-dense secretory granules (Moran et al., 1982a). The observation that Bowman’s glands in Blarina contained both alcian blue-positive and PAS-positive secretory materials support the idea that these two cells are producing different secretory products, one of which is probably an acid mucopolysaccharide and the other a neutral mucopolysaccharide. The presence of acid and neutral mucopolysaccharides in Bowman’s glands has been previously reported in mice (Cuschieri and Bannister, 1975), rats (Bojsen-Moller, 1964) and guinea pigs (Ruseva, 1972). However, in rabbits (Zinnin, 1964), hogs (Herberhold, 1968) and monkeys (Shantha and Nakajima, 1970) only neutral mucopolysaccharides were found. Since the basic ultrastructure of the two types of Bowman’s gland cells differed significantly, especially in the amounts and types of endoplasmic reticulum, it is unlikely that these cells are the same cell type at different points in the secretory cycle.
3.4 Comparative Studies

According to studies by Larochelle and Baron (1989a, b), in *Blarina*, 63% of the nasal cavity is devoted to the olfactory epithelium. *Blarina* had the largest surface area of olfactory epithelium and most developed epithelium of the four shrews studied which included two terrestrial species, *Sorex cinereus* and *S. fumeus*, and one semi-aquatic species, *S. palustris*. In *Sorex minutus*, also a terrestrial shrew, the olfactory epithelium covered approximately 61.7% of the nasal cavity. The size of the olfactory mucosa is reduced in the semi-aquatic Insectivora compared to terrestrial forms. *Neomys fodiens*, the European watershrew, and *S. palustris* were reported to have the least-developed olfactory epithelium of the shrews studied. Only 41% of the nasal cavity of *N. fodiens* is devoted to the olfactory epithelium (Larochelle and Baron, 1989a; Baron and Stephan, 1996). Any differences in the olfactory epithelium and olfactory development are probably positively related to the ecological niche (Larochelle and Baron, 1989a, b). The statistical results from these various shrews indicate that *Blarina* has a highly-developed olfactory epithelium.

3.5 Conclusion

The results of this detailed ultrastructural study of the olfactory epithelium and mucosa in the two species of these *Blarina* confirm that these small mammals have a very well-developed olfactory epithelium and mucosa and suggest that these semi-fossorial shrews rely greatly upon olfaction in their daily activities.
CHAPTER IV

MAIN OLFACTORY BULB

4.1 Introduction

Main olfactory bulb (MOB) microscopic structure has been studied in numerous macrosmatic mammalian species including the platypus (Smith, 1895, 1896; Hines, 1929), marsupial shrew opossum (Herrick, 1921); hedgehog (Lopez-Mascaraque et al., 1986, 1989; Brodmann, 1999; Brinon et al., 2001), mole (Crosby and Humphrey, 1939a; Johnson, 1957b), opossum (Herrick, 1892; Herrick, 1924; Switzer and Johnson, 1977; Chuah et al., 1997), rat (Reese and Brightman, 1970; Orona et al., 1983; Orona et al., 1984; Scheibel and Scheibel, 1975; Mair and Gesteland, 1982; Mair et al., 1982; Struble and Walters, 1982; Doucette, 1984; Switzer et al., 1985; Morizumi et al., 1995; Kosaka et al., 1998), mouse (Hirata, 1964; Burd, 1980; Carson, 1984; Graziadei and Graziadei, 1986; Dellovade et al., 1998), rabbit (Young, 1934, 1936; Allison and Warwick, 1949; Allison, 1953a; Broadwell, 1975a; Mori et al., 1983; Ojima et al., 1984; Katoh et al., 1994; Yilmazer-Hanke et al., 2000), cat (McCotter, 1912; Ryu, 1980; Wahle et al., 1990; Sztamska and Goetzen, 1997), dog (Read, 1908; McCotter, 1912), and the ferret (Lockard, 1985). The MOB of the microsmatic mammals has also been studied and includes the monkey (Alonso et al., 1998; Bassett et al., 1992) and human (Sarnat and Netsky, 1981; Bhatnagar et al., 1987; Ohm et al., 1991; Shipley and Reyes, 1991; Sztamska and Goetzen, 1997).
4.2 Results

4.2.1 MOB Gross Anatomy

In the lateral view (Fig. 13), the MOB of the short-tailed shrew was relatively large. The cerebral hemispheres were smooth with few gyri. The olfactory tubercle and piriform cortex were also large.

4.2.2 Light Microscopy Overview

In the sagittal plane of section (Fig. 14), the large MOB dominated the rostral telencephalon. The well-developed anterior olfactory nucleus filled the olfactory peduncle. The rostral-most region of the frontal cortex projected over the caudal parts of the MOB. The small accessory olfactory bulb (AOB) was positioned at the caudal edge of the dorsal region of the MOB. In coronal sections, the large MOB was oval and contained six concentric layers (Fig. 15) including the olfactory nerve layer (ONL), glomerular layer (GL), external plexiform layer (EPL), mitral cell layer (MCL), internal plexiform layer (IPL), and granule cell layer (GrL). At rostral levels, the ONL was thickest on the ventral and medial surfaces. At a more caudal level (Fig. 16), the olfactory ventricle was deep within the GrL and was partially surrounded by the subependymal zone (see Fig. 72). At its most caudal level, the crescent-shaped MOB was found on the ventro-medial surface of the cerebral hemisphere adjacent to the pars posterior of the AON and the piriform cortex (Fig. 17) and was ventral to the piriform cortex and rostral lateral ventricle. The laminar organization of the MOB remained constant from rostral to caudal levels (Fig. 18).
Fig. 13. Line drawing of the lateral view of the cortex and cerebellum. Large olfactory structures include the main olfactory bulb (MOB), olfactory tubercle (OT), and piriform cortex (PC). Smooth cerebral hemisphere (CH), cerebellum (CE).
Fig. 14. Light micrograph of the sagittal plane of the main olfactory bulb. Pars dorsalis (PD) and pars lateralis (PL) of the anterior olfactory nucleus, frontal cortex (FC), accessory olfactory bulb (arrow), pars externa of the AON (curved arrow), main olfactory bulb (MOB), olfactory ventricle (arrowhead), nucleus accumbens (NA), caudate-putamen (C-P). 40 μm frozen section, sagittal plane, cresyl-violet stain. 5 mm = 165 μm.
Fig. 15. Light micrograph of the rostral main olfactory bulb. The six concentric layers include the olfactory nerve layer (ONL), glomerular layer (GL), external plexiform layer (EPL), mitral cell layer (curved arrow), internal plexiform layer (arrowhead) and granule cell layer (GrL). 40 μm frozen section, coronal plane, cresyl-violet stain. 5 mm = 165 μm.

Fig. 16. Light micrograph of the mid-caudal main olfactory bulb. The open olfactory ventricle (OV) with adjacent subventricular zone (arrow) and granule cell layer (GrL). 40 μm frozen section, coronal plane, cresyl-violet stain. 5 mm = 165 μm.
Fig. 17. Light micrograph of the caudal main olfactory bulb. The caudal-most MOB is crescent-shaped. Pars posterior (PP) of the anterior olfactory nucleus and piriform cortex (PC) are ventral to the frontal cortex (FC). 40 μm frozen section, coronal plane, cresyl-violet stain. 5 mm = 250 μm.

Fig. 18. Light micrograph of the main olfactory bulb layers. Olfactory nerve layer (ONL), glomerular layer (GL), external plexiform layer (EPL), mitral cell layer (arrow), internal plexiform layer (arrowhead) and granule cell layer (GrL). 40 μm frozen section, coronal plane, cresyl-violet stain. 5 mm = 50 μm.
4.2.3 Light Microscopy of Olfactory Nerve Layer (ONL)

The ONL (Fig. 19) consisted of olfactory receptor axons and ensheathing cells. Two types of ensheathing cells, a light and dark cell, were scattered throughout the ONL. These ensheathing cells contained oval nuclei and a darkly-stained nucleolus was often present. Ensheathing cell nuclei measured up to 6 \( \mu \text{m} \) in diameter along the long axis.

4.2.4 Light Microscopy of Glomerular Layer (GL)

The GL (Fig. 20) consisted of spherical glomeruli and the surrounding periglomerular region. Neurons in the periglomerular region included periglomerular cells (PGC) and short-axon cells (SAC). In the ventral regions, glomeruli were stacked two or three thick. Glomeruli diameters ranged up to 150 \( \mu \text{m} \). Capillaries were common between glomeruli. In 1 \( \mu \text{m} \) epoxy resin sections the glomeruli consisted of darkly-stained olfactory receptor axons intermingled with pale-staining dendrites. PGCs encircled the glomeruli with fewer cells on the side facing the ONL. PGCs were most numerous adjacent to the external plexiform layer often forming a cap-like structure on the glomeruli. These cells were oval or spherical often with a prominent nucleolus and measured up to 11 \( \mu \text{m} \) in diameter. The second cell type, the SAC was spherical and more lightly stained than the adjacent periglomerular cells. These cells were less common than the PGCs and measured up to 13 \( \mu \text{m} \) in diameter. A third cell, the external tufted cell (ETC) (Fig. 21) had a large spherical euchromatic nucleus and measured up to 17 \( \mu \text{m} \). The ETC cell bodies were located in the superficial EPL. The apical dendrites of the tufted cells extended into the GL and terminated within the glomeruli.
Fig. 19. Light micrograph of the olfactory nerve layer (ONL). Unmyelinated axons (A),
dark ensheathing cell (arrow), light ensheathing cell (curved arrow), glomerular layer
(GL). 1 μm epoxy resin section, Richardson stain. 5 mm = 7 μm.

Fig. 20. Light micrograph of the glomerular layer. Glomeruli with dark axons (arrow)
and lighter areas of dendrites (arrowhead). Periglomerular cells (P), short-axons cells
(S). Olfactory nerve layer (ONL) with ensheathing cells. 1 μm epoxy resin section,
Richardson stain. 5 mm = 4 μm.
4.2.5 Light Microscopy of External Plexiform Layer (EPL)

The EPL (Fig. 22) consisted of tufted cells, numerous capillaries, myelinated axons, and dendritic profiles. Large tufted cells were scattered throughout the entire EPL and measured up to 19 µm in diameter. The tufted cells of the EPL were similar in morphology to the external tufted cells. Pale staining cross-sectional and longitudinal profiles of tufted cell and mitral cell dendrites were common within the EPL. Some of these dendrites projected to the GL and some ran horizontally in the EPL. Small myelinated axons were common.

4.2.6 Light Microscopy of Mitral Cell Layer (MCL)

The MCL consisted of a single row of very large cells with rounded nuclei (Fig. 23). An occasional granule cell was adjacent to the mitral cells. Mitral cells measured up to 29 µm in diameter. The euchromatic nucleus often had a distinct nucleolus. Abundant pale cytoplasm surrounded the nucleus. The cell body tapered, forming the apical dendrite which extended into the EPL. Basal dendrites extended laterally and diagonally into the EPL. Mitral cell axons extended down through the granule cell layer.

4.2.7 Light Microscopy of Internal Plexiform Layer (IPL)

The IPL (Fig. 23) was located between the MCL and the granule cell layer. This layer consisted of mitral and tufted cell axons and dendrites of granule cells.
Fig. 21. Light micrograph of the external tufted cell. External tufted cells (E) are located deep to the glomerular layer (GL). The external tufted cell apical dendrite (arrowhead) extends into a glomerulus (arrow). External plexiform layer (EPL). 1 μm epoxy resin section, Richardson stain. 5 mm = 6 μm.

Fig. 22. Light micrograph of the external plexiform layer. Pale dendritic profiles (D), tufted cell (T) with apical dendrite (arrowhead), and myelinated axons (arrow) of the EPL. 1 μm epoxy resin section, Richardson stain. 5 mm = 9 μm.
4.2.8 Light Microscopy of Granule Cell Layer (GrL)

The GrL (Fig. 24) had elongated clusters of cells oriented parallel to the MCL alternating with regions of neuropil. Bands of granule cell bodies were typically 3-12 nuclei thick. Two types of granule cells were present in these bands. One type, a dark granule cell contained a spherical nucleus with a darkly stained nucleolus. These dark granule cells were more numerous than the light granule cell. The nuclear diameter measured up to 6 μm. The second type of granule cell had a spherical nucleus with small patches of heterochromatin that resulted in an overall lighter staining intensity than the dark granule cells. These light granule cell nuclei measured up to 8 μm in diameter. The surrounding neuropil in these bands consisted mostly of pale staining dendrites. Between the bands the neuropil contained small myelinated axons, dendrites, and capillaries.

4.2.9 Transmission Electron Microscopy of ONL

Unmyelinated olfactory receptor axons (Fig. 25) in cross section and longitudinal section filled the ONL and contained mitochondria, neurofilaments, and microtubules. The axon diameters ranged from 0.2-0.4 μm with an average diameter of 0.2 μm. Ensheathing cell processes formed incomplete septa partially surrounding groups of axons. Dark ensheathing cells (Fig. 25) had more heterochromatin than the light ensheathing cells (Fig. 26). Ensheathing cell nuclei were typically oval. Ensheathing cell cytoplasmic organelles commonly included numerous mitochondria, rough endoplasmic reticulum (RER), free ribosomes, microtubules, a prominent Golgi apparatus, and intermediate filaments. Lipofuscin granules were often common in the cytoplasm of both types of ensheathing cells.
Fig. 23. Light micrograph of the mitral cell layer. External plexiform layer (EPL) with numerous pale dendritic profiles (curved arrow), apical dendrite (D) of a tufted cell, mitral cell (M) of the mitral cell layer, mitral cell apical dendrite (arrowhead), proximal part of a basal dendrite (arrow), internal plexiform layer (IPL). 1 μm epoxy resin section, Richardson stain. 5 mm = 10 μm.

Fig. 24. Light micrograph of the granule cell layer. The granule cell layer (GrL) with distinct bands of light granule cells (arrowhead), dark granule cells (arrow), and myelinated axons (curved arrow). 1 μm epoxy resin section, Richardson stain. 5 mm = 5 μm.
Fig. 25. TEM micrograph of dark ensheathing cells. Dark ensheathing cell with lipofuscin granules (arrowhead). Cross-sections and longitudinal-sections of unmyelinated olfactory receptor cell axons (A). 5 mm = 0.8 μm.

Fig. 26. TEM micrograph of a light ensheathing cell. Light ensheathing cell with lipofuscin granules (arrowhead). Cross-sections and longitudinal-sections of unmyelinated olfactory receptor cell axons (A). 5 mm = 0.8 μm.
4.2.10 Transmission Electron Microscopy of GL

Periglomerular cells were the most common neurons in the periglomerular region of the GL (Fig. 27). The periglomerular cell nucleus was often indented and had scattered patches of heterochromatin. The nucleolus was typically eccentric. The thin layer of cytoplasm contained numerous ribosomes arranged in rosette structures, small amounts of RER, and Golgi profiles. Lipofuscin granules were often present in periglomerular neurons. Asymmetrical dendro-somatic synapses between external tufted cell dendrites and periglomerular cell somas were common (Fig. 27). The second cell type of the periglomerular region, the short-axon cell was less numerous than the periglomerular cell and often had an indented nucleus (Fig. 28). The cytoplasm contained a few profiles of RER and large profiles of Golgi apparatus. Free ribosomes and mitochondria were scattered throughout the cytoplasm. A third neuronal cell type, the external tufted cell was identified by its large size, euchromatic nucleus, and abundant electron-lucent cytoplasm (Fig. 29). These neurons were typically located at the interface between the EPL and GL. The tufted cell apical dendrite projected into the GL. Long parallel arrays of RER and Golgi stacks were common in the perinuclear cytoplasm. Rosettes of ribosomes and mitochondria were uniformly scattered throughout the tufted cell cytoplasm. These dendrites were smooth in outline and contained mitochondria and microtubules.

Glomeruli consisted of many profiles of electron-dense receptor cell axons and terminals with pale dendrites of mitral, tufted, and periglomerular cells (Fig. 30). These axon terminals were all ultrastructurally similar and contained numerous electron-lucent vesicles, microtubules and mitochondria. The electron-lucent dendrites from all three
Fig. 27. TEM micrograph of periglomerular cells (PG). Dendritic profiles (D), myelinated axons (curved arrow), and a dendro-somatic synapse (arrowhead) between the primary dendrite of an external tufted cell and a periglomerular soma are observed within the periglomerular region. Nuclear filaments (arrow) are occasionally observed in periglomerular neurons. 5 mm = 0.6 μm.
Fig. 28. TEM micrograph of the short-axon cell (SA). The cytoplasm contains numerous mitochondria (M) and large profiles of Golgi apparatus (G). Nuclear pseudoinclusions formed by indentations of the nuclear envelope (arrowheads). Glomerulus (GL).

5 mm = 0.6 μm.
Fig. 29. TEM micrograph of the external tufted cell. The cytoplasm of the external tufted cell contains a well-developed Golgi apparatus (G). Numerous large dendritic profiles (D) are common within the periglomerular region. Note the periglomerular cells (PG) in close proximity to the tufted cell. 5 mm = 1 μm.
Fig. 30. TEM micrograph of axons and dendrites in the glomeruli. The glomeruli of the GL typically consist of electron-dense unmyelinated receptor cell axons (A) and terminals intermingled with electron-lucent dendritic profiles (D). Dendro-dendritic synapses (arrow) and axo-dendritic synapses (arrowhead) are common. 5 mm = 0.5 μm.
neuron types were similar and contained scattered mitochondria, microtubules, and profiles of SER. Dendro-dendritic and axo-dendritic synapses were common in the glomeruli. Symmetrical and asymmetrical synapses were common. Symmetrical synapses were characterized by a thin synaptic density between the two membranes (Figs. 31, 32). Pleomorphic electron-lucent vesicles were grouped near the presynaptic density. Asymmetrical synapses had a more prominent synaptic density that extended farther into the postsynaptic structure (Fig. 32). The presynaptic structure was identified by the presence of predominantly electron-lucent spherical vesicles (Figs. 33, 34).

4.2.11 Transmission Electron Microscopy of EPL

The EPL consisted of a dense plexus of primary and secondary dendrites of mitral and tufted cells intermingled with dendrites of granule cells (Figs. 35, 36). The dendrites of the mitral and tufted cells and granule cells were ultrastructurally indistinguishable from one another. These dendrites contained many microtubules and mitochondria. Dendro-dendritic synapses were numerous within the EPL and often three to five synaptic contacts were common along one dendritic profile (Fig. 35). The dendro-dendritic synapses were both asymmetrical and symmetrical (Figs. 35, 36). On rare occasions myelinated tufted cells and dendrites were identified in the EPL. The tufted cell bodies were partially surrounded by a thin myelin sheath (Figs. 37, 38). In addition, myelinated dendrites were also noted in the EPL (Fig. 39).
Fig. 31. TEM micrograph of a dendro-dendritic synapse. This dendro-dendritic symmetrical synapse in the glomerulus contains electron-lucent pleomorphic vesicles (arrow) in the presynaptic region. The synaptic density (arrowhead) is thin between the two dendritic membranes. Numerous cross-sections of microtubules (M) are present in adjacent dendrites. 5 mm = 0.2 μm.

Fig. 32. TEM micrograph of a reciprocal synapse. Electron-lucent pleomorphic vesicles in the terminal region (arrowhead), spherical vesicles in the dendrite of a mitral/tufted cell (arrow) in the glomerulus. 5 mm = 0.2 μm.
Fig. 33. TEM micrograph of a axo-dendritic synapse. This axo-dendritic asymmetrical synapse (arrowhead) in the glomerulus contains electron-lucent spherical vesicles (arrows) in the presynaptic region. 5 mm = 0.2 μm.

Fig. 34. TEM micrograph of a dendro-dendritic synapse. Asymmetrical synapse in the glomerulus contains electron-lucent spherical vesicles (arrows) in the presynaptic region. 5 mm = 0.2 μm.
Fig. 35. TEM micrograph of dendro-dendritic synapses (arrow). Spherical electron-lucent vesicles are characteristic of these asymmetrical synapses (arrowhead). Darker structures forming synapses appear to be granule cell dendrites and dendritic spines. Mitral/tufted cell dendrites (D) in the external plexiform layer. 5 mm = 0.3 μm.

Fig. 36. TEM micrograph of pleomorphic vesicles. Pleomorphic electron-lucent vesicles (arrowhead) are characteristic of symmetrical dendro-dendritic synapses in the external plexiform layer. 5 mm = 0.1 μm.
Fig. 37. TEM micrograph of a myelinated neuron. A tufted cell soma with a thin myelin sheath (arrow) in the external plexiform layer. 5 mm = 0.4 μm.
Fig. 38. TEM micrograph of the thin myelin sheath. The sheath typically consists of a few thin lamellae (arrowhead). 5 mm = 0.1 μm.

Fig. 39. TEM micrograph of a myelinated dendrite (D). Myelinated axons (AX) in the external plexiform layer. 5 mm = 0.4 μm.
4.2.12 Transmission Electron Microscopy of MCL

The mitral cells (MC), the largest cells of the MOB, had a euchromatic nucleus surrounded by abundant cytoplasm (Fig. 40). The nucleus typically contained a large dense nucleolus. RER was often organized into parallel arrays. Numerous mitochondria, free ribosomes, and Golgi stacks were common. The initial segments of MC dendrites typically contained many microtubules and mitochondria.

4.2.13 Transmission Electron Microscopy of IPL

The IPL (Fig. 41) contained numerous cross-sections of mitral and tufted cell myelinated axons. It was not possible to distinguish between the tufted and mitral cell axons or the afferent fibers, although since the MC were the largest cells, it is likely that the largest axons were from MC. The axons were densely clustered together forming a thin IPL.

4.2.14 Transmission Electron Microscopy of GrL

The GrL (Figs. 42, 43) of the MOB consisted of granule cells and the neuropil of dendrites of granule cells and numerous unmyelinated axons. Occasional myelinated axons were also present in the GrL. The dendrites were electron-lucent and contained microtubules and mitochondria. The axon terminals were typically densely packed predominantly with small, spherical electron-lucent vesicles. Asymmetric synapses with spherical vesicles were present common (Fig. 42). The second subdivision contained the spherical granule cells (Fig. 43). The light and dark granule cells were clustered together. Both types of granule cells had a spherical or oval nucleus. The dark granule cell was more numerous than the light granule cell. The lighter granule cell nucleus had less heterochromatin and appeared more electron-lucent. Both cell types had a narrow band
Fig. 40. TEM micrograph of a mitral cell. Nucleus (N) with prominent nucleolus. Golgi apparatus (G), mitochondria (M) and rough endoplasmic reticulum (arrow).

5 mm = 0.8 μm.
Fig. 41. TEM micrograph of myelinated axons. Myelinated axons (arrow) of mitral and tufted cells are interspersed with those of afferent fibers in the internal plexiform layer. 5 mm = 0.5 µm.

Fig. 42. TEM micrograph of the neuropil of the granule cell layer. The neuropil consists of electron-lucent dendrites (D) intermingled with axon terminals with spherical vesicles (arrow) and asymmetric synapses (arrowhead). 5 mm = 0.1 µm.
Fig. 43. TEM micrograph of dark and light granule cells. The dark granule cell has electron-dense patches of heterochromatin (arrowheads) evenly distributed throughout the nucleus. The lighter granule cell nucleus typically has a more electron-lucent nucleus with a distinct nucleolus (arrow). 5 mm = 0.5 μm.
of cytoplasm containing free ribosomes and small amounts of RER.

4.3 Discussion

The typical mammalian MOB organization with the six concentric layers (Young, 1934, 1936; Crosby and Humphrey, 1939a; Jeserich, 1945; Lauer, 1945; Allison, 1953b; Andres, 1970; MacLeod, 1977; Lockard, 1985; Lopez-Mascaraque et al., 1986; Greer, 1991; McLean and Shipley, 1992; Nickell and Shipley, 1992; Kratskin, 1995; Shipley and Smith, 1995; Shipley et al., 1995; Butler and Hodos, 1996; Eisthen, 1997; Alonso et al., 1998; Kosaka et al., 1998) surrounding the olfactory ventricle was also found in *Blarina*. Furthermore, the various typical cell types of the MOB (Herrick, 1924; Jeserich, 1945; Johnson, 1957b; Andres, 1970; Brooke and Pinching, 1975; Doucette, 1984; Greer, 1991; Bassett et al., 1992; Dellovade et al., 1998) were also common in the MOB of *Blarina*. The synaptic patterns of the mammalian MOB (Hirata, 1964; Andres, 1970; Price and Powel, 1970a, b, c; Pinching and Powell, 1971a, b, c; Brooke and Pinching, 1975; Shepherd and Greer, 1990; Greer, 1991; Scott and Harrison, 1991; Shipley and Reyes, 1991; Bassett et al., 1992; Nickell and Shipley, 1992) were also present in *Blarina*. One main difference observed in the MOB in *Blarina* pertained to its caudal extent. The MOB tissue extended significantly more caudally which caused the shrew AON to be displaced dorsal and lateral compared to rodents (Burns, 1982). There were several additional differences in the MOB of *Blarina* compared to other small macrosmatic mammals. These differences will be elaborated upon in the following sections.
4.3.1 Olfactory Nerve Layer

Olfactory receptor neurons have been reported to continually die throughout the life of the adult animal and are replaced by mitosis and differentiation of basal cells. Axons of new receptors grow through the cribriform plate of the ethmoid bone and enter the ONL on the surface of the MOB. The ONL has been reported to be thickest on the anterior and ventral surfaces in the rabbit (Young, 1936; Yilmazer-Hanke et al., 2000) and rat (Doucette, 1984). In Blarina, the ONL was the thickest on the medial and ventral surfaces of the MOB. Crosby and Humphrey (1939a) reported that the ONL was thickest on the medial, ventral, and lateral surfaces of the shrew. Within the glomeruli, the receptor cell axons form synapses with mitral and tufted cell dendrites (Alison, 1953a; Shepherd and Greer, 1990; Kratskin, 1995; Brinon et al., 2001). In the rabbit, these axons are reported to have average diameters of 0.6 μm (Yilmazer-Hanke et al., 2000). In Blarina the axons were slightly smaller than the axon diameter in the rabbit and ranged from 0.2-0.4 μm in diameter.

According to Doucette (1984), glial cells of the rat ONL include ensheathing cells and astrocytes. Ensheathing cells have ultrastructural properties of both fibrous astrocytes and Schwann cells and are found in association with the olfactory nerves (Barber and Lindsay, 1982; Chuah et al., 2000; Imaizumi et al., 2000; Keyvan-Fouladi et al., 2002; Wang et al, 2003). The ensheathing cell processes, like Schwann cell processes, wrap around groups of unmyelinated olfactory receptor axons and are thought to provide support for the olfactory axon regeneration (Doucette, 1984; Pixley, 1992; Kafitz and Greer, 1998; Barnett et al., 2000). In the rat, the nuclei of ensheathing cells and astrocytes are ultrastructurally similar. However, the ensheathing cell cytoplasm
appears more dense than typical astrocyte cytoplasm. In addition, intermediate filaments in the ensheathing cells are fewer in number and are scattered throughout the cytoplasm whereas in the fibrous astrocyte, these intermediate filaments are organized into bundles and are more distinct. Based upon the morphological observation of scattered intermediate filaments, absence of filament bundles, and the observed ensheathing of axonal bundles, the glial cells of Blarina ONL were identified as ensheathing cells. Doucette (1984) observed only one type of ensheathing cell in the rat, but in Blarina, light and dark ensheathing cells were common.

4.3.2 Glomerular Layer

The GL of Blarina was similar to the mammalian MOB GL (Mori et al., 1983; Nickell and Shipley, 1992; Katoh et al., 1994; Kosaka et al., 1998; Kasowski et al., 1999; Treloar et al., 1999; Kim and Greer, 2000). Glomerular shape and size varies within and among mammalian species. Generally, the glomerulus is oval, spherical, or pear-shaped. Table 2 is a summary of glomerular diameter variations among some mammals.

<table>
<thead>
<tr>
<th>Mammal species</th>
<th>Diameter (μm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shrew</td>
<td>-150</td>
<td>Present study</td>
</tr>
<tr>
<td>Rat</td>
<td>50-120</td>
<td>Pinching and Powell, 1971b</td>
</tr>
<tr>
<td>Rat</td>
<td>40-300</td>
<td>Royet et al., 1989</td>
</tr>
<tr>
<td>Rat</td>
<td>100-200</td>
<td>MacLeod, 1977</td>
</tr>
<tr>
<td>Rabbit</td>
<td>50-200</td>
<td>Katoh et al., 1994</td>
</tr>
<tr>
<td>Macaque</td>
<td>70-100</td>
<td>Alonso et al., 1998</td>
</tr>
</tbody>
</table>
The glomerular diameter in *Blarina* was similar to glomerular diameter in other macrosmatic mammals and measured up to 150 µm. The glomerular diameter in *Blarina* was significantly larger than the glomerular diameter in the microsmatic macaque monkey (Alonso et al., 1998).

Two types of synapses have been reported in mammalian MOB glomeruli. Symmetrical synapses are identified by thin electron-dense membrane thickenings between the two synaptic membranes with pleomorphic vesicles in the presynaptic region. Asymmetrical synapses have thicker synaptic densities that typically extend farther into the postsynaptic region. Spherical vesicles are associated with the asymmetrical synapses (Price and Powell, 1970a, b, d; Pinching and Powell, 1971a, b, c).

Table 3 is a summary of the common synaptic patterns identified within the rat GL. Cell types to the left of the arrow are presynaptic. Cells to the right of the arrow are postsynaptic.

<table>
<thead>
<tr>
<th>Type of synapse</th>
<th>Symmetrical</th>
<th>Asymmetrical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dendro-dendritic</td>
<td>PGC→M/T</td>
<td>M/T→PGC</td>
</tr>
<tr>
<td></td>
<td>PGC→PGC</td>
<td></td>
</tr>
<tr>
<td>Axo-dendritic</td>
<td>SAC→PGC</td>
<td>ONA→PGC</td>
</tr>
<tr>
<td></td>
<td>PGC→M/T</td>
<td>ONA→M/T</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ETC→PGC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CF→PGC</td>
</tr>
<tr>
<td>Dendro-somatic</td>
<td></td>
<td>M/T→PGC</td>
</tr>
</tbody>
</table>

PGC=periglomerular cell; M/T=mitral/tufted cells; SAC=short-axon cell; ONA=olfactory nerve axon; ETC=external tufted cell; CF=centrifugal fibers
The periglomerular cell dendritic processes have been reported to extend into one or more glomeruli whereas the tufted and mitral cell apical dendrites project to a single glomerulus (Kratskin, 1995). The axon terminals in the glomeruli originate from various sources including olfactory receptor cells, SACs, PGCs, and centrifugal fibers (Price, 1968; Pinching and Powell, 1971a, b, c; Getchell and Shepherd, 1975; Kosaka et al., 1998). The axons of the short-axon cells extend for a short distance throughout the GL, projecting a distance of only one to three glomeruli; periglomerular cell axons extend across three to five glomeruli. These axons do not typically extend beyond the boundaries of the GL, but remain within the GL surrounding the glomeruli. The receptor cell axons originate from the olfactory epithelial receptor cells and terminate with the glomeruli. Centrifugal axon fibers approach the MOB via the lateral olfactory tract and terminate in the glomeruli (Price, 1968; Pinching and Powell, 1971a, b, c; Kratskin, 1995). The synaptic patterns in the MOB glomeruli of *Blarina* were comparable to those reported from other mammals, especially in the rat as described by Pinching and Powell (1971a, b, c). Symmetrical and asymmetrical dendro-dendritic and axo-dendritic synapses were observed in *Blarina*. Spherical, electron-lucent vesicles in the presynaptic terminals were characteristic of the asymmetrical synapse whereas pleomorphic, electron-lucent vesicles were associated with the symmetrical synapses. In addition, Pinching and Powell (1971a, b, c) reported symmetrical somato-dendritic synapses originating on the periglomerular cells and terminating on mitral and tufted cell dendrites. These synapses were less frequent than those listed in Table 3 and rarely occurred. This type of synapse was not observed in *Blarina*. 

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
The surrounding periglomerular region of the GL has been defined as the interstices between individual glomeruli, between the glomeruli and the ONL, and between the glomeruli and the EPL. This region contains several types of neuronal somata. Periglomerular cells are the most common neuron of the GL and range in diameter in the rat from 5-8 μm (Pinching and Powell (1971a, c). Brinon and coworkers (2001) report similar cellular diameters of 5-9 μm in the hedgehog, a close relative of *Blarina*. Periglomerular cells in *Blarina* were slightly larger than those of the rat and the hedgehog and measured up to 11 μm in diameter.

A second cell type of the GL is the short-axon cell. These cells range in diameter from 8-12 μm in the rat and are less numerous than periglomerular and external tufted cells (Pinching and Powell, 1971a). In *Blarina*, short-axon cells were comparable to the rat and measured up to 13 μm. The short-axon cells in *Blarina* were also less numerous than the periglomerular cells and the external tufted cells.

A third cell type, the external tufted cell is located within the periglomerular region or in close proximity to the glomerular region (Pinching and Powell, 1971a; Haberly and Price, 1977; Mori et al., 1983; Orona et al., 1984). External tufted cells send a primary dendrite into the GL and several secondary dendrites into the superficial region of the EPL (Kratskin, 1995). The external tufted cell diameter ranges from 10-15 μm in the rat (Pinching and Powell, 1971a) and 15-19 μm in the hedgehog (Brinon et al., 2001). In *Blarina*, the external tufted cells measured up to 17 μm and were comparable to those of the rat and hedgehog MOB in their diameter.
4.3.3 External Plexiform Layer

The EPL is characterized by numerous tufted cells scattered throughout a neuropil consisting of primary and secondary dendrites from tufted cells and mitral cells and peripheral dendrites from granule cells (Young, 1934; Shepherd, 1966; Druga, 1980; Mori et al., 1983; Greer, 1991; Kratskin, 1995). Two of the three types of tufted cells are located in the EPL. These cells are called middle tufted cells and internal tufted cells. Middle and internal tufted cells have also been called displaced mitral cells or outwandered mitral cells in older reports (Young, 1934; Crosby and Humphrey, 1939a). The middle tufted cells are located within the mid-regions of the EPL whereas the internal tufted cells are deep within the EPL just above the MCL. The third type of tufted cell, which has been previously discussed, is the external tufted cell located at the border between the GL and the EPL. Each type of tufted cell is similar in staining intensity and morphology (Pinching and Powell, 1970a; Mori et al., 1983; Schneider and Scott, 1983; Kratskin, 1995; Nakajima et al., 1996; Gil-Carcedo et al., 2000) but may vary in diameter. According to Haberly and Price (1977), tufted cells become smaller in size as the distance from the MCL increases. In the hedgehog (Brinon et al., 2001), tufted cells of the EPL ranged in diameter from 16-24 μm and in the rat (Pinching and Powell, 1971a) tufted cell diameters ranged from 18-25 μm. In Blarina, tufted cells measured only up to 19 μm in diameter. Additionally, Kosaka and Kosaka (1999) have identified in the laboratory shrew Suncus murinus a cell type referred to as the tasseled cell that resembles tufted cells of the EPL. The tasseled cell soma is located within the middle region of the EPL and its dendrites extend towards the GL. At the border of the GL and the EPL is a region that contains small glomerular-like structures called nidi. The nidi resemble the glomeruli of the GL. Surrounding the nidi are specialized cells called...
perinidal cells which are like the periglomerular cells. The dendrites of the tasseled cells branch into a tuft-like structure and synapse on perinidal cells within the nidi. Kosaka and Kosaka (1999) have suggested that many of the outwandered mitral cells noted by Crosby and Humphrey (1939a) should probably be recognized as tasseled cells. Immunocytochemical studies were used to identify the nidus, perinidal cells, and tasseled cells. The current project on Blarina focused on the ultrastructure of the olfactory system by light and transmission electron microscopy so cellular identification based upon immunocytochemical methods were not within the scope of this research. The presence or absence of tasseled neurons in Blarina can not be confirmed nor rejected based upon morphological observations.

Myelinated neuronal somas and dendrites were observed within the EPL of Blarina. Historically, these types of structures have not been a frequent finding. However, investigators have discovered these myelinated structures in various layers of the MOB of other mammals. Tigges and Tigges (1980) reported myelinated neuronal somata within the periglomerular region in the squirrel monkey MOB. These myelinated somata were identified as external tufted cells based upon their morphology and their position within the superficial regions of the EPL. In addition, myelinated periglomerular cells have been observed within the GL. Tigges and Tigges (1980) further reported occasional myelinated somata of granule cells deep within the GrL. Segments of myelinated dendrites are also noted within the periglomerular region and the EPL. In the mouse, Burd (1980) reported myelinated mitral/tufted cell dendrites in the EPL. The function of these myelinated structures remains unknown. Tigges and Tigges (1980) hypothesize that myelination may be the result of changes associated with the aging
process and not due to pathological conditions. In *Blarina*, based upon position and ultrastructural characteristics, the myelinated somata appeared to be tufted cells and the myelinated dendrites appeared to be external tufted cell primary dendrites. The function of these myelinated structures remains unknown. Their rarity suggests that they do not play a major role in MOB function.

The synapses within the EPL are relatively simple. Reciprocal dendro-dendritic synapses between mitral and tufted cell dendrites and granule cell dendrites are common (Pinching and Powell, 1971a, b, c; Mouradian and Scott, 1988). In dendro-dendritic reciprocal synapses, it has been suggested that mitral and tufted cell dendrites are excitatory to the granule cells, whereas the granule cells are inhibitory to the mitral and tufted cell dendrites. These reciprocal synapses function to inhibit and/or filter incoming olfactory information from the olfactory mucosa (Brooke and Pinching, 1975; Brunjes et al., 1982; Kishi et al., 1982; Mori et al., 1983; Mouradian and Scott, 1988; Shipley and Reyes, 1991). The reciprocal synapse consists of an asymmetrical and a symmetrical synapse. The asymmetrical synapse originates on mitral and tufted cell secondary dendrites and terminates on the granule cell dendrites. Electron-lucent spherical vesicles are reported in the presynaptic dendritic region. The symmetrical synapse originates on the granule cell dendritic process and terminates on the mitral and tufted cell dendrites. The vesicles associated with the presynaptic dendrite were reported to be electron-lucent pleomorphic vesicles (Price and Powel, 1970a, b). In *Blarina*, asymmetrical synapses were identified by the presence of predominantly spherical electron-lucent vesicles in large mitral and tufted cell dendrites. Also, symmetrical synapses were observed originating from the granule cell dendrite and terminating onto the dendrites of the mitral
and tufted cells. Small, electron-lucent pleomorphic vesicles were clustered in the presynaptic dendritic processes of these symmetrical synapses. The asymmetrical synapses with predominantly spherical vesicles and symmetrical synapses with pleomorphic vesicles observed in Blarina were similar to the synapses and vesicles reported in the rat (Price and Powel, 1970a, b; Pinching and Powell, 1971a, b, c).

4.3.4 Mitral Cell Layer

Mitral cells and tufted cells are the efferent neurons of the MOB (Greer, 1991; Cajal, 1995; Kratskin, 1995; Kosaka et al., 1998). These cells share many morphological characteristics. However, mitral cells are distinguished from tufted cells based on the position of their somata. In most mammals, mitral cells are confined to a monolayer whereas tufted cells have been observed scattered within the EPL (Orona et al., 1984). Both cell types have a single apical dendrite that extends without branching throughout the EPL into a glomerulus where synaptic contact is made with olfactory nerve axons and periglomerular cell axons and dendrites. The secondary dendrites of mitral and tufted cells extend in a tangential pathway, parallel to the MCL, into the EPL and synapse onto granule cell dendrites. The axons of mitral and tufted cells exit the MOB and form the lateral olfactory tract (Lohman and Mentink, 1969; Druga, 1980; Orona et al., 1984; Greer, 1991; Nickell and Shipley, 1992; Kosaka et al, 1998). In Blarina, the MCL consisted of a monolayer of densely packed mitral cells. This monolayer organizational feature is not found in the olfactory bulbs of some Monotremes, specifically the platypus or the echidna. The lack of a monolayer has also been reported in some reptiles, such as the python (Switzer and Johnson, 1977). In birds, a monolayer was reported in the emu and kiwi, but was absent in the chicken (Switzer and Johnson, 1977). In the hedgehog,
the mitral cells are not organized in the typical monolayer but are displaced within the EPL and IPL (Lopez-Mascaraque et al., 1986). In the macaque monkey (Alonso et al., 1998), mitral cells are also dispersed throughout the EPL and do not form a monolayer. It is interesting that a mitral cell monolayer was observed in Blarina but is absent in the hedgehog. The functional significance of mitral cells organized in a monolayer versus a generalized displacement remains unclear. The lack of a mitral cell monolayer appears to be more common in primitive vertebrates including the chameleon (Anolis carolinensis), copperhead (Agkistrodon mokaseri), and sparrow (Passer domesticus) (Crosby and Humphrey, 1939a). In primates, the lack of a monolayer may be part of the regression of the olfactory system.

4.3.5 Internal Plexiform Layer
The IPL of Blarina was similar to the typical mammalian IPL and contains relatively few neuronal somata, but had numerous collaterals of mitral and tufted cell myelinated axons and peripheral dendrites of granule cells (Allison, 1953a; Shepherd, 1966; Orona et al., 1984; McLean and Shipley, 1992; Kratskin, 1995). In addition to these efferent fibers, afferent axons from the forebrain coming into the MOB also pass through the IPL (Scott and Harrison, 1991).

4.3.6 Granule Cell Layer
Granule cells of Blarina were organized into bands of cells within a neuropil of dendrites of granule cells and collaterals of axons from mitral and tufted cells. Shipley and Ennis (1996) also reported that granule cells in other mammals typically form bands of cells. Afferent projections to the MOB originate from several sources including the ipsilateral
and contralateral anterior olfactory nucleus and the ipsilateral horizontal limb of the diagonal band. These afferent fibers make asymmetrical synapses onto the granule cells (Young, 1934; Cragg, 1962; Price, 1968; Price and Powell, 1970c; Davis et al., 1978; Macrides et al., 1981; Nickell and Shipley, 1992; Cajal, 1995). In Blarina, afferent axons also made asymmetrical synapses on granule cell dendritic processes. These synapses typically occurred within the neuropil surrounding the bands of granule cells. Granule cells have been reported to lack axons (Pinching and Powell, 1971a; MacLeod, 1977; Mair et al., 1982). Two types of dendritic processes, the peripheral and deep dendrites extend from the granule cells. These peripheral processes are relatively thick, terminate in the EPL and enter into reciprocal synapses with mitral and tufted cell dendrites (Price and Powell, 1970a, b; Orono et al., 1983). From the deep side of the perikarya, 1-4 dendrites extend toward deeper regions within the GrL, extending away from the MCL.

The diameter of granule cells appears to be relatively consistent among mammals. In the hedgehog, these cells ranged from 6-8 µm in diameter (Lopez-Mascaraque et al., 1986; Brinon et al., 2001) and in the rat, granule cells ranged from 6-10 µm in diameter (Mair et al., 1982). Struble and Walters (1982) have identified two populations of granule cells in the rat, a dark and a light granule cell. Dark granule cells were 6-11 µm in diameter, and the light granule cells were 7-13 µm in diameter. Struble and Walters (1982) concluded that although light granule cells are the larger cell of the two types, the dark granule cells are more numerous than the light granule cells. These two populations of granule cells were also identified in Blarina. The dark granule cells in Blarina measured up to 6 µm in diameter and were more numerous than the light granule cells which were larger and measured up to 8 µm in diameter. A subset of granule cells,
termed atypical granule cells has been reported in the cat (Wahle et al., 1990). These cells, located in the MCL and EPL lack the peripheral dendrite common to typical granule cells. Rather, two or more dendrites extend in the EPL in an oblique fashion. Other atypical granule cells are located at the border between the GL and EPL. Cells found within the GL are identified along the surface of the glomeruli themselves, or in the spaces between the structures. In Blarina, the two populations of dark and light granule cells were located only within the GrL. In addition, Shepherd and Greer (1990) noted short axon cells in the GrL of the rat. These cells were not observed in the GrL of Blarina.

The olfactory ventricle appeared deep within the GrL of Blarina and was similar to other macrosmatic mammals including the hedgehog (Lopez-Mascaraque et al., 1986, 1989; Valverde et al., 1989) and mole (Johnson, 1957b), marsupial mole (Smith, 1895), opossum (Herrick, 1892; Herrick, 1924), rabbit (Young, 1934; Broadwell, 1975b; Romfh, 1982), and the cat (Ryu, 1980; Kinney, 1982; Wahle et al., 1990). In some mammals, such as the mink (Jeserich, 1945), the olfactory ventricle is small. In the platypus (Hines, 1929), rat (Burns, 1982), and mouse (Crosby and Humphrey, 1939a), the ventricle is occluded. In humans, the ventricle is patent only in fetal life (Sarnat and Netsky, 1981) and only in rare cases has an open olfactory ventricle remained within the MOB (Roy et al., 1987).

4.4 Comparative Studies

According to Stephan and coworkers (1991), the MOB in Blarina makes up approximately 12% of the telencephalon volume. In Neomys fodiens, a semiaquatic shrew, the MOB makes up only 9% of the telencephalon. This reduced MOB size is
expected since olfaction is not thought to be as important in detection of aquatic prey. By comparison, in the macrosmatic gray wolf, *Canus lupus*, the MOB is 19% of the total brain volume and in the red fox, *Vulpes vulpes*, also a macrosmatic mammal, the MOB is 18% of the total brain volume (Gittleman, 1991). Interestingly, the MOB in Megachiroptera is well-developed and comparable to the MOB in the Insectivora (Baron et al., 1996). In Megachiroptera, approximately 8% of the telencephalon is devoted to the MOB. In Microchiroptera, only 5% of the telencephalon is devoted to the MOB. In microsmatic mammals, the MOB percentage is significantly less. For example, in Primates, on average, only 2.4% of the telencephalon is devoted to the MOB. In simians, the percentage is greatly reduced to only 0.2% (Stephan et al., 1991). The large telencephalon volume devoted to olfaction in *Blarina* suggests that the olfactory system is very well-developed compared to most other Insectivora, as well as other mammals.

Stephan and coworkers (1991) plotted the volume of the MOB against body weight in a double logarithmic scale resulting in a size index. In *Blarina*, the MOB size index was reported to be 89. In *Neomys fodiens*, a semiaquatic shrew, the MOB index was 59, a significantly smaller index. Other members of the Insectivora were found to be comparable to *Blarina*. In *Sorex araneus*, the common shrew, the MOB size index was 81 and in *S. minutus*, the pygmy shrew, the MOB index was 72. The MOB size index for the house shrew, *Suncus murinus* was 80. In other Insectivora, the MOB size index of *Talpa europaea*, the European mole, was only 74 whereas in the hedgehog *Erinaceus europaeus*, the index measured 93. Thus it appears that the MOB in *Blarina* is larger than most Insectivora.
4.5 Conclusion

Based upon the high percentage of telencephalon volume and the large MOB size index, it appears that the MOB of *Blarina* is well-developed. In addition, the morphological observations of the MOB of *Blarina* confirm the hypothesis that this olfactory system structure differs from that of other small mammals and its high degree of development suggests that olfaction is of primary importance in these shrews.
CHAPTER V
ACCESSORY OLFATORY BULB

5.1 Introduction

The vomeronasal olfactory system, consisting of the vomeronasal organ (VNO), accessory olfactory bulb (AOB), and the amygdala, has been investigated in many mammals including the duck-billed platypus (Smith, 1895), shrew (Crosby and Humphrey, 1939a; Stephan, 1965), mole (Johnson, 1957), opossum (McCotter, 1912; Halpern et al., 1998a), bat (Frahm, 1981; Acharya et al., 1998; Bhatnagar and Meisami, 1998), mouse (Barber and Raisman, 1978, 1979; Weruaga et al., 2001; Taniguchi and Kaba, 2001), guinea pig (Sugai et al., 1997), hamster (Davis et al., 1978; Meredith, 1980), ferret (Lockard, 1985), mink (Jeserich, 1945; Salazar et al., 1998), rat (Burns, 1982; Rosselli-Austin et al., 1987; Roos et al., 1988; Takami and Graziaedi, 1991), rabbit (Young, 1934; 1936; Romfh, 1982), dog (Adams and Wiekamp, 1984; Salazar et al., 1994; Nakajima et al., 1998), cat (Wahle et al., 1990; Won et al., 1997), and the human (Moran et al., 1991; Smith et al., 1998). The AOB has been proposed to function in mammalian reproductive behaviors (Wysocki and Meredith, 1991; Jansen et al., 1998; Dudley and Moss, 1999; Brennan, 2001; Korsching, 2001; Kondo et al., 2003).

The location of the AOB varies among mammals. Crosby and Humphrey (1939a, b) described the location of the AOB as either an “in folding” into the MOB, such as in the mouse, rat, and red squirrel, or as an “eminence” on the surface of the MOB. In the guinea pig (McCotter, 1912; Meisami and Bhatnagar, 1998), the extremely large AOB is described an eminence on the dorsolateral surface of the caudal MOB. In the rabbit (Young, 1936) and opossum (McCotter, 1912) the AOB also is reported to be an
eminence on the dorsomedial surface of the caudal MOB. In the mole and shrew, it appears that the AOB has developed inside the MOB (Crosby and Humphrey, 1939a, b). In *Blarina*, the AOB was located on the dorsomedial surface of the caudal MOB and appeared to develop inside the MOB.

5.2 Results

5.2.1 Light Microscopy Overview

In a parasagittal section, the AOB was located in the dorsal part of the caudal MOB. The MOB external plexiform layer, mitral cell layer, and granule cell layer were adjacent to the rostral AOB (see Fig. 14). Brain landmarks found at the most rostral level of the AOB (Fig. 44) included the pars lateralis and pars externa of the anterior olfactory nucleus and the olfactory ventricle. At a more caudal level of the AOB (Fig. 45), the ventral pars lateralis became crescent-shaped and the middle section of the pars externa disappeared. The layering of the shrew AOB were much less distinct than that of the MOB and consisted of the vomeronasal nerve layer (VNL), glomerular layer (GL), a combined external plexiform layer/mitral-tufted cell layer (EP/M-TCL), internal plexiform layer (IPL), and granule cell layer (GrL) (Fig. 46). The AOB was shaped like an inverted triangular with the base adjacent to the inferior border of the frontal cortex. A myelinated fiber tract passed through the IPL of the AOB (Fig. 47).
Fig. 44. Light micrograph of the rostral accessory olfactory bulb (arrowhead). Frontal cortex (FC), pars externa (PE) of the anterior olfactory nucleus, olfactory ventricle (arrow), pars lateralis (PL) of the anterior olfactory nucleus. 40 μm frozen section, coronal plane, cresyl-violet stain. 5 mm = 165 μm.

Fig. 45. Light micrograph of the caudal accessory olfactory bulb (arrowhead). MOB granule cell layer (GrL), olfactory ventricle (arrow), pars lateralis (PL), pars externa (PE), frontal cortex (FC). 40 μm frozen section, coronal plane, cresyl-violet stain. 5 mm = 165 μm.
Fig. 46. Light micrograph of the laminar organization of the accessory olfactory bulb. Layers outlined by dashed lines include the vomeronasal nerve layer (arrow), glomerular layer (GL), external plexiform/mitral-tufted cell layer (E), internal plexiform layer (IPL), granule cell layer (G). Landmarks include the frontal cortex (FC), external plexiform layer of the main olfactory bulb (EPL/MOB), granule cell layer of the main olfactory bulb (GrL/MOB), pars externa of the anterior olfactory nucleus (PE) and pars lateralis of the anterior olfactory nucleus (PL). 40 μm frozen section, coronal plane, cresyl-violet stain. 5 mm = 40 μm.
5.2.2 Light Microscopy of Vomeronasal Nerve Layer (VNL)

The vomeronasal nerve layer (VNL) consisted of bundles of unmyelinated axons and ensheathing cells (Fig. 48). The ensheathing cell nuclei were oval and had distinct nucleoli. Ensheathing cell nuclei measured up to 8 µm in diameter and were interspersed among the unmyelinated fibers. In the MOB, two types of ensheathing cells, the dark and light ensheathing cells, were common. In the AOB, only one type of ensheathing cell was observed. The AOB ensheathing cells were similar to the dark ensheathing cells of the MOB. Numerous capillaries were scattered throughout the VNL.

5.2.3 Light Microscopy of Glomerular Layer (GL)

The glomerular layer (GL) consisted of acellular glomeruli and a surrounding neuropil with periglomerular cells (Fig. 49). The glomeruli were not distinct spherical structures as in the MOB. The AOB glomeruli consisted of darkly stained vomeronasal axons and pale staining dendrites. Periglomerular cells had an oval nucleus with a distinct nucleolus and some heterochromatin. Periglomerular cell nuclei measured up to 8 µm in diameter. Periglomerular cells were scattered throughout the neuropil of the GL and did not form the cap-like structures seen in the GL of the MOB.

5.2.4 Light Microscopy of External Plexiform /Mitral-Tufted Cell Layer (EP/M-TCL)

The EP/M-TCL consisted of a neuropil of axons and dendritic profiles, capillaries, and mitral/tufted cells. The mitral/tufted cells (Fig. 50) were scattered uniformly throughout the EP/M-TCL. The large euchromatic nuclei measured up to 9 µm and had distinct nucleoli. Few dendrites were observed extending from these mitral/tufted cell perikarya.
Fig. 47. Light micrograph of an overview of the accessory olfactory bulb. Vomeronasal nerve layer (curved arrow), glomerular layer (GL), external plexiform layer/mitral-tufted cell layer (E), internal plexiform layer/lateral olfactory tract (arrowhead), granule cell layer (GrL), MOB granule cell layer (G/MOB), MOB external plexiform layer (E/MOB). 1 μm epoxy resin section, Richardson stain. 5 mm = 28 μm.

Fig. 48. Light micrograph of the vomeronasal nerve layer (VNL). Ensheathing cells (arrow), MOB external plexiform layer (EPL/MOB). 1 μm epoxy resin section, Richardson stain. 5 mm = 5 μm.
Fig. 49. Light micrograph of the glomerular layer. Periglomerular cells (arrowhead) are interspersed between the glomeruli. Glomeruli consist of dark unmyelinated vomeronasal axons (A) and pale staining dendrites (arrow). AOB glomeruli are not distinct spherical structures like MOB glomeruli. 1 μm epoxy resin section, Richardson stain. 5 mm = 5 μm.

Fig. 50. Light micrograph of the external plexiform/mitral-tufted cell layer (EP/M-TCL). Numerous mitral/tufted cells (arrowheads), glomerular layer (GL). 1 μm epoxy resin section, Richardson stain. 5 mm = 6 μm.
AOB mitral/tufted cells appeared to have less cytoplasm and less prominent dendrites than the MOB mitral or tufted cells.

5.2.5 Light Microscopy of Internal Plexiform Layer (IPL)

Ventral to the EP/M-TCL was a thick fiber tract consisting of densely packed myelinated axons (Fig. 51) that formed part of the lateral olfactory tract. The IPL separated the EP/M-TCL from the underlying region that consisted of the AOB granule cell layer and a subdivision of the anterior olfactory nucleus.

5.2.6 Light Microscopy of Granule Cell Layer (GrL)

The granule cell layer (Fig. 51) consisted of a cluster of granule cells interspersed among pale staining dendrites and was located ventral to the IPL. Granule cells were not organized in the banding pattern characteristic of the MOB GrL. The granule cell nuclei were oval with scarce amounts of heterochromatin and measured up to 8 µm in diameter. In the MOB, light and dark granule cells were common. The AOB granule cell was similar to the light granule cell of the MOB. Interspersed between the granule cells were pale staining dendrites and small myelinated axons. Adjacent to the granule cells were large euchromatic nuclei of the pars lateralis neurons of the anterior olfactory nucleus.

5.2.7 Transmission Electron Microscopy of VNL

The unmyelinated vomeronasal axons of the VNL (Fig. 52) ranged from 0.2-0.6 µm in diameter. Ensheathing cells (Fig. 53) had an elongated nucleus with densely stained heterochromatin. A thin layer of perinuclear cytoplasm contained mitochondria, RER, scattered free ribosomes, and lipofuscin granules. The AOB ensheathing cells were similar to the dark ensheathing cells of the MOB.
Fig. 51. Light micrograph of the granule cell layer. Granule cells (arrowhead) of the AOB granule cell layer (GrL), internal plexiform layer (IPL), pars lateralis neurons of the anterior olfactory nucleus (arrow). 1 μm epoxy resin section, Richardson stain. 5 mm = 6 μm.

Fig. 52. TEM micrograph of the vomeronasal nerve layer. Unmyelinated fibers of the vomeronasal nerve layer (VNL), external plexiform layer of the main olfactory bulb (EPL/MOB). 5 mm = 0.5 μm.
Fig. 53. TEM micrograph of ensheathing cells of the vomeronasal nerve layer.

Ensheathing cell nuclei contain electron-dense heterochromatin. Lipofuscin granules (arrow), unmyelinated axons (A). 5 mm = 0.4 μm.
5.2.8 Transmission Electron Microscopy of GL

The glomeruli of the GL contained vomeronasal receptor cell axon terminals and electron-lucent periglomerular cell and mitral/tufted cell dendrites. Mitral/tufted cell dendrites and periglomerular cell dendrites were morphologically identical. The axon terminals were electron-dense and contained numerous microtubules and mitochondria. The dendrites were significantly more pale than the axon terminals and contained microtubules and mitochondria (Fig. 54). In the glomeruli, asymmetrical axo-dendritic synapses between vomeronasal axons and mitral/tufted cell and periglomerular cell dendrites contained small spherical vesicles in the axon terminals (Fig. 55). Dendro-dendritic synapses between mitral/tufted cells and periglomerular cells were also observed in the glomerular layer. These synapses were identified by the vesicles contained within the dendrites. Pleomorphic vesicles were common in symmetrical synapses (Fig. 56) and predominantly spherical vesicles characterized the asymmetrical synapses (Fig. 57). Few periglomerular cells were observed in the GL. Periglomerular cell nuclei (Fig. 58) were typically indented and heterochromatic. The thin layer of cytoplasm contained RER, free ribosomes and numerous mitochondria. These cells were more commonly found at the border between the GL and the underlying EP/M-TCL. The AOB periglomerular cells were not located between adjacent glomeruli nor did these cells form the cap-like structure noted within the GL of the MOB.

5.2.9 Transmission Electron Microscopy of EP/M-TCL

The EP/M-TCL contained numerous mitral/tufted cells surrounded by a neuropil of axons, synaptic terminals, and dendrites. Mitral/tufted cells (Fig. 59) were identified by their spherical euchromatic nuclei and abundant cytoplasm. The cytoplasm contained
Fig. 54. TEM micrograph of a glomerulus. The glomerulus consists of electron-dense axon terminals (A) and electron-lucent dendrites (D) of mitral/tufted cells and periglomerular cells. Mitochondria (arrow) are common in the dendrites. 5 mm = 0.3 µm.

Fig. 55. TEM micrograph of a axo-dendritic synapse. Axo-dendritic asymmetrical (arrows) synapses originate on vomeronasal axons and terminate on dendrites of mitral/tufted cells and periglomerular cells. 5 mm = 0.1 µm.
Fig. 56. TEM micrograph of a dendro-dendritic synapse. Dendro-dendritic symmetrical synapses in the glomerular layer are identified by the pleomorphic electron-lucent vesicles (arrowheads). 5 mm = 0.1μm.

Fig. 57. TEM micrograph of a dendro-dendritic synapse. Dendro-dendritic asymmetrical synapses (arrow) in the glomerular layer are identified by the spherical electron-lucent vesicles (arrowheads). 5 mm = 0.1μm.
Fig. 58. TEM micrograph of a periglomerular cell. Nuclear indentations (arrow) are common in the periglomerular cells. The thin layer of perinuclear cytoplasm contains numerous free ribosomes and mitochondria. 5 mm = 0.8 μm.

Fig. 59. TEM micrograph of mitral/tufted cells. The cytoplasm typically contains RER (arrow) and numerous mitochondria (arrowhead). 5 mm = 0.9 μm.
mitochondria, RER profiles, a well-developed Golgi apparatus, and scattered free ribosomes. Thinly myelinated axons were common adjacent to mitral/tufted cells. Numerous dendro-dendritic synapses between mitral/tufted cell dendrites and granule cell dendrites were observed. Symmetrical dendro-dendritic synapses contained electron-lucent pleomorphic vesicles within the terminal region (Fig. 60) and asymmetrical dendro-dendritic synapses contained spherical electron-lucent vesicles (Fig. 61).

5.2.10 Transmission Electron Microscopy of IPL
The internal plexiform layer (Fig. 62) was characterized by densely packed thinly myelinated axons of the LOT. Mitochondria were scattered throughout the axoplasm. Pale granule cell dendrites were interspersed in the neuropil of the IPL.

5.2.11 Transmission Electron Microscopy of GrL
The GrL was ventral to the IPL and contained few granule cells (Fig. 63). These cells typically had oval nuclei and distinct electron-dense nucleoli. The thin layer of perinuclear cytoplasm contained numerous free ribosomes, sparse amounts of RER, and numerous mitochondria. The AOB granule cells were similar to the light granule cells of the MOB. The surrounding neuropil consisted of electron-lucent dendrites and myelinated axons.

5.3 Discussion
Among the vertebrates, the AOB differs in its shape and size. The shape of the AOB varies from hemispheric (opossum), oval (rat, rabbit) to cone-shaped (hedgehog). In the mole, the AOB appears wedge-shaped and in the red squirrel, the AOB is crescent-shaped (McCotter, 1912; Crosby and Humphrey, 1939a; Jeserich, 1945; Johnson, 1957;
Fig. 60. TEM micrograph of pleomorphic vesicles. Dendro-dendritic symmetrical synapses in the external plexiform/mitral-tufted cell layer contain pleomorphic electron-lucent vesicles (arrowhead). 5 mm = 0.1 μm.

Fig. 61. TEM micrograph of spherical vesicles. Dendro-dendritic asymmetrical synapses in the external plexiform/mitral-tufted cell layer are identified by the presence of spherical electron-lucent vesicles (arrowhead) in the terminal region. 5 mm = 0.1 μm.
Fig. 62. TEM micrograph of the internal plexiform layer. The layer consists of densely packed myelinated axons (AX) of the lateral olfactory tract and dendrites of granule cells. $5 \, \text{mm} = 0.5 \, \mu\text{m}$.

Fig. 63. TEM micrograph of the granule cell layer. Granule cell with a prominent nucleolus (arrow), myelinated axons of the lateral olfactory tract (AX). $5 \, \text{mm} = 0.5 \, \mu\text{m}$. 

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
The shape of the AOB in *Blarina* was similar to the mole and was wedge-shaped or triangular-shaped. Additionally, the size and development of the AOB varies among vertebrates. In some reptiles, including the garter snake, copperhead, water moccasin, and Gila monster, the AOB appeared to be highly-developed. However, in other reptiles, such as the green anole (*Anolis carolinensis*), horned lizard (*Phrynosoma cornutum*), and American alligator (*American Mississipiensis*) no AOB was observed (Crosby and Humphrey, 1939b). In mammals, the size and presence of the AOB varies significantly. In the primitive monotremes, including the duck-billed platypus and ant-eaters, the AOB was reported to be small. In the marsupial kangaroo and opossum, the AOB is well-developed with distinct layers. In the carnivorous cat and dog, the AOB is relatively small compared to the large, well-developed MOB. Significant size variations have been reported in rodents. In the guinea pig, the AOB is extremely large. The AOB is well-developed and relatively large in the red squirrel, rat, mouse, and hamster. Among bats, the size of the AOB varies greatly. In some species of bats the AOB is large, yet in other bat species, the AOB is small. In the Insectivora, the AOB varies in size and development. In shrews and moles, the AOB was reported to be small and poorly-developed, but in the hedgehog, the AOB appears to be large with distinct layers. In primates, significant variations have been reported. The AOB is present in prosimians and appears large and well-developed. Among the simians, the AOB is well-developed in the New World monkeys, but in the Old World monkeys, including the macaque, apes, and humans, the AOB is not present (McCotter, 1912, Crosby and Humphrey, 1939a, b; Frahm, 1980, 1981; Meisami and Bhatnagar, 1998). According to Meisami and Bhatnagar (1998), there are marked variations in the size and
development of the AOB among numerous vertebrates and these differences may represent differences in sensory discrimination.

Typically, a large AOB has a high degree of lamination with well-defined layers that are named according to the corresponding laminar organization in the MOB. Matsuoka and coworkers (1998) identified the VNL, GL, mitral/tufted, GrL, and the olfactory tract layers in the hamster which has a well-developed AOB. In the rat, the layers are also well-developed and easily identified (Meisami and Bhatnagar, 1998). In mammals with a small AOB, distinct layers are more difficult to identify (McLean and Shipley, 1992; Meisami and Bhatnagar, 1998; Salazar et al., 1998). In the dog, only the GL and EPL with mitral/tufted cells are readily identified (Salazar et al., 1994). In the mink, a distinct GL, mitral/tufted cell layer, and GrL have been identified (Salazar et al., 1998). In some species, AOB layers are thin and poorly-differentiated and discrimination of separate layers is impractical so several layers are often combined. Meisami and Bhatnagar (1998) reported a well-developed, large AOB in the hedgehog. However, in other reports (Shipley et al., 1995; Salazar and Quinteiro, 1998; Brinon et al., 2001), the EPL, MCL, and the IPL of the hedgehog AOB are not distinct so these layers have been combined into a single EPL/MCL/IPL complex. In *Blarina*, the small AOB was similar to other mammals with a small AOB. The poorly-developed layers were not easily identified and several layers were combined. The layers of the *Blarina* AOB included the VNL, GL, external plexiform/mitral-tufted cell layer, IPL, and GrL.

5.3.1 Vomeronasal Nerve Layer

Vomeronasal nerves belong to the peripheral nervous system and are formed by bundles of unmyelinated axons that originate from sensory receptor cells located within the
vomeronasal (Jacobson’s organ) epithelium. These nerves pass through the cribriform plate of the ethmoid bone, bypass the MOB, and spread out over the surface of the AOB to form a distinct nerve layer (Meisami and Bhatnagar, 1998). Axon bundles are separated from adjacent bundles by ensheathing cells (Barber et al., 1978; Vaccarezza et al., 1981; Fraher, 1982; Raisman, 1985; Wysocki and Meredith, 1991; Dudley and Moss, 1999). The ensheathing cells of the VNL are similar to the ensheathing cells found in the ONL of the MOB (Fraher, 1982). The VNL ensheathing cells in Blarina were similar to the typical mammalian ensheathing cell. In addition, the unmyelinated axons of the VNL are reported to be approximately 0.2 μm in diameter in the rat (Raisman, 1985). The vomeronasal axon diameters in Blarina were larger and ranged from 0.3-0.6 μm in diameter.

5.3.2 Glomerular Layer

The GL in Blarina was less distinct than the GL of the MOB and consisted of vomeronasal receptor axons and dendrites of mitral/tufted cells and periglomerular cells. The AOB glomeruli in Blarina were fewer in number compared to the MOB and were not spherical structures as in the MOB. Therefore, an accurate measurement of glomerular diameter was not possible. In addition, there were less periglomerular cells in the AOB. The periglomerular cell in Blarina measured up to 8 μm in diameter and was significantly smaller than the periglomerular cells in the rat that measure 7-20 μm in diameter (Takami et al., 1992). Because these cells did not form the cap-like structures common on the MOB glomeruli, the AOB glomeruli were not well-delineated. Poorly-defined AOB glomeruli have been reported in other mammals including the hamster (Matsuoka et al., 1998), guinea-pig (Sugai et al., 1997), mole (Crosby and Humphrey,
1939a), and in the rat (Takami and Graziadei, 1990, 1991). Short-axon cells are common in the GL of the mammal MOB but these cells are not common in the AOB.

The neuronal circuitry of the AOB is simple in comparison to the MOB (Scalia and Winans, 1975, 1976; Meredith, 1980; Lehman and Winans, 1982; Clancy et al., 1984; Brennan et al., 1990; Brennan, 2001). The glomerulus is the site of the first synaptic contact within the vomeronasal system (Halpern et al., 1995; Halpern et al., 1998b). Direct projections from the VNO sensory axons synapse on the apical dendrites of mitral/tufted cells and periglomerular cells within multiple glomeruli of the GL (Crosby and Humphrey, 1939b; Frahm and Bhatnagar, 1980; Imamura et al., 1985; Wysocki and Meredith, 1991; Jia and Halpern, 1997; Matsuoka et al., 1997, 1998; Dudley and Moss, 1999; Sugai et al., 1999). In the mouse (Matsuoka et al., 1998) and in the rat (Jia et al., 1999) axo-dendritic asymmetrical synapses between the vomeronasal axons and mitral/tufted cell dendrites have been reported. The dendrites of mitral/tufted cells and periglomerular cell are reported to be morphologically similar. In Blarina, similar asymmetrical axo-dendritic synapses were observed. The vomeronasal nerve axon terminal contained numerous electron-lucent spherical vesicles. In addition, dendro-dendritic asymmetrical and symmetrical synapses were commonly observed between periglomerular cells and mitral/tufted cells. These asymmetrical and symmetrical synapses were similar to the synapses in the MOB.

5.3.3 External Plexiform/Mitral-Tufted Cell Layer

In Blarina, the monolayer of mitral cells forming the mitral cell layer commonly found in the MOB was not present in the AOB. Rather, the mitral/tufted cells appeared scattered throughout the EPL. Therefore, this layer was referred to as the external
plexiform/mitral-tufted cell layer. Poorly-defined layers have been reported in other mammals. In the dog (Salazar et al., 1994), the EPL and MCL are indistinguishable from each other. In several bat species with a large AOB, the EPL is compressed between a thickened GL and MCL (Frahm and Bhatnagar, 1980).

Mitral/tufted cells are reported to be the second order output neurons of the AOB (Takami and Graziadei 1990, 1991; Bonfanti et al., 1997; Jia et al., 1999). Golgi studies show that the AOB mitral/tufted cells have up to six apical dendrites that project to several glomeruli whereas in the MOB, a single apical dendrite enters only one glomerulus (Jia and Halpern, 1997; Meisami and Bhatnagar, 1998; Keverne, 1999; Brennan, 2001). Mitral/tufted cells of the rat AOB are large and range from 15-24 μm (Takami and Graziadei, 1990, 1991). In Blarina, the AOB mitral/tufted cells are significantly smaller than the rat and measured only up to 9 μm in diameter.

Dendro-dendritic reciprocal synapses have often been reported in the EPL. Asymmetrical dendro-dendritic synapses originate on the mitral/tufted cell basal dendrites and the symmetrical dendro-dendritic synapses originate on the granule cell dendrites (Brennan et al., 1990; Matsuoka et al., 1997, 1998; Jia et al., 1999; Taniguchi and Kaba, 2001). Similar reciprocal dendro-dendritic synapses were also present in Blarina. Asymmetrical dendro-dendritic synapses were readily identified by the presence of spherical vesicles and symmetrical dendro-dendritic synapses were identified by pleomorphic vesicles.

**5.3.4 Internal Plexiform Layer/Myelinated Fiber Tract**

In the MOB, the IPL is well-developed and consists of mitral/tufted cell axons and centrifugal afferents. In mammals with a large AOB such as the rat, a distinct IPL is
common and lies above the lateral olfactory tract which consists of MOB and AOB mitral/tufted cell myelinated axons (Price, 1991; Meisami and Bhatnagar, 1998). In mammals with a less-developed AOB, such as some bat species (Frahm and Bhatnagar, 1980), the IPL is thin and not distinct. In the mouse, the thin IPL is enclosed within the tract of myelinated axons and is not well-differentiated from the tract (Salazar et al., 2001). In different species, this myelinated fiber tract may run either through the AOB between the IPL and the GrL or under the GrL of the AOB which separates the AOB from the MOB (Meisami and Bhatnagar, 1998). According to Meisami and Bhatnagar (1998), this myelinated fiber tract runs through the AOB in shrew, hedgehog, and mole. Other mammals with the through pattern include the tenrecs, bushbaby, guinea pig, rat, and mouse (Switzer et al., 1980). Interestingly, the tract in the opossum runs under the AOB and deviates from the through pattern seen in other small mammals. The under pattern appears to be prevalent in numerous carnivores such as the cat, lion, dog, bear, and weasel. In Blarina, the IPL and myelinated axons were intermixed and ran through the AOB, a situation similar to that in the hedgehog and mole.

5.3.5 Granule Cell Layer

The GrL is deep to the layer of myelinated fibers (Barber and Raisman, 1978) and has been difficult to identify in some species including bats (Frahm and Bhatnagar, 1980; Kinney, 1982) and dogs (Salazar et al., 1994; Meisami and Bhatnagar, 1998). In Blarina, the GrL was ventral to the IPL/myelinated fiber tract and consisted of a small clustering of granule cells. In the MOB, granule cells are grouped together in islets (Meisami and Bhatnagar, 1998), but in the AOB, the granule cells are too few to form islets. In the cat (Wahle et al., 1990) and in the dog, (Nakajima et al., 1998), AOB granule cells are
approximately 10 μm in diameter. In the rat, AOB granule cells have been reported to range from 7-15 μm in diameter (Rosselli-Austin, 1987; Takami et al., 1992). Struble and Walters (1982) described by light microscopy two populations of AOB granule cells in rats. One type, the dark granule cell measured 9 μm in diameter and the second type, the light granule cell measured 10 μm in diameter. The light granule cells are the predominant granule cell of the rat AOB. In Blarina, only light granule cells were observed. These cell diameters were smaller than the light granule cells in the rat and measured only 8 μm in diameter.

5.4 Comparative Studies

To compare the AOB of various mammals, the volume of the AOB is plotted against the body weight in double logarithmic scales (Stephan et al., 1991). According to Stephan and coworkers (1991), the AOB has been found in all Insectivora investigated. The size of the AOB ranges from very small to rudimentary. The AOB size index for Blarina is 71 which is lower than in other shrews. For example, Sorex fumeus, the smoky shrew, has an index of 93. Other shrews with varying habitats have a significantly higher AOB index compared to Blarina and include Sorex araneus (157), Suncus etruscus (129), and Neomys fodiens (144). Two Insectivora are closely related to Blarina, the European hedgehog, Erinaceus europaeus, and the European mole, Talpa europaea, have AOB indices of 205 and 143 respectively. Based upon these indices, it appears that no relationship can be established between AOB size and habitat of the Insectivora. Stephan and coworkers (1982) reported that the average AOB size index for prosimians was 354 and the average for simians was 56. Stephan and coworkers (1991) also reported a significantly larger AOB in the prosimians and a much smaller AOB in the simians. This
gradual decrease results in the absence of the AOB in humans. Since the AOB functions in sexual behaviors, Stephan and coworkers (1982) suggest that the visual system has compensated for the reduction of the AOB in those mammals, including humans, with a small, poorly-developed AOB. There appears to be significant variations in the size and development of the AOB in the vertebrates. In some reptiles, the AOB is large but in others, such as the alligator, the AOB is absent. In the various birds investigated by Crosby and Humphrey (1939a), no AOB was reported. In mammals, the primitive monotremes have a small AOB. A small AOB has also been reported in various carnivores. In simians, a large and a small AOB have been reported. In marsupials, rodents, and prosimians, the AOB appears to be large and well-developed. Although there are exceptions, it appears that primitive mammals have a small, poorly-developed AOB and the evolutionary advanced mammals have a large, well-developed AOB.

5.5 Conclusion

The AOB in Blarina is a small, wedge-shaped structure similar to that of the mole AOB. Compared to the rat which has a well-developed AOB, the cells of the AOB in Blarina are consistently smaller than the cells of the rat AOB. The layers of the Blarina AOB are also less-differentiated than the corresponding layers of the MOB. In addition, the glomeruli of the GL are poorly defined. The low AOB index in Blarina and the morphological observations by light and electron microscopy indicate that the AOB in Blarina is a poorly-developed structure that is similar to other Insectivora but differs significantly from other small mammals such as the rat. Since this accessory olfactory system in Blarina is poorly developed while the main olfactory system is highly

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
developed, these small mammals may be depending upon their main olfactory system and
the trigeminal complex for olfactory acuity and not the accessory system.
CHAPTER VI

ANTERIOR OLFACTORY NUCLEUS

6.1 Introduction

Herrick (1924) first described the gray matter in the olfactory peduncle, just caudal to the MOB as a nucleus and termed the structure the “nucleus olfactorius anterior.” This area, currently known as the anterior olfactory nucleus (AON) is now recognized actually to be a cortical structure, not a nucleus. This bilayer structure consists of several divisions, each made up of an outer plexiform layer and an inner layer densely packed with pyramidal cells (Shipley and Ennis, 1996). The plexiform layer consists of pyramidal cell dendrites and axons as well as terminals of the lateral olfactory tract axons (LOT). The AON is a major relay in the olfactory pathway. In addition to input from the MOB via the LOT, the AON also receives inputs from the ipsilateral and contralateral AON, the piriform cortex, and olfactory tubercle (Valverde et al., 1989; Shipley and Ennis, 1996). The AON is divided into subdivisions that are named based upon their location with respect to the anterior commissure and the olfactory ventricle. Exact boundaries between the subdivisions have not been precisely defined and controversy regarding the specific names applied to the various subdivisions remains a current problem (Bayer, 1986; Barbado et al., 2001). For example, in the ferret, the AON is divided into the dorsal, dorsomedial, ventromedial, ventral, lateral, externa, and, the most caudal, the posterior subdivisions (Lockard, 1985). In the rat, six subdivisions have been identified which include the externa, lateralis, dorsalis, medialis, ventralis and posterior subdivision (Bayer, 1986; Garcia-Ojeda et al., 1998). Some researchers have combined the ventralis and posterior subdivisions in the rat into the ventroposterior subdivision (Reyher et al.,
1988; Barbado et al., 2001). In the hedgehog, a close relative of the shrew, the AON subdivisions include the bulbaris, principalis which includes the dorsalis, lateralis, medialis, ventralis, the externa, and posterior. The pars bulbaris consists of AON fibers coursing through the anterior commissure at the level of the olfactory ventricle (Valverde et al., 1989).

The AON has been investigated in numerous mammalian species including the platypus (Hines, 1929), opossum (Herrick, 1924; Meyer, 1981; Shammah-Lagnado and Negrao, 1981), hedgehog (De Carlos et al., 1989; Valverde et al., 1989; Radtke-Schuller and Künzle, 2000), mole (Johnson, 1957b), hamster (Ferrer, 1969; Devor, 1976; Davis and Macrides, 1981; Schoenfeld and Macrides, 1984), guinea pig (Johnson, 1957a), ferret (Lockard, 1985), rabbit (Young, 1934; Broadwell, 1975b; Mori et al., 1979; Ojima et al., 1984), rat (Haberly and Price, 1978b; Bennett, 1968; Shafa and Meisami, 1977; De Olmos et al., 1978; Luskin and Price, 1983a, b; Alheid et al., 1984; Friedman and Price, 1984; Scott et al., 1985; Reyher et al., 1988; Brown and Brunjes, 1990; Garcia-Ojeda et al., 1998; Barbado et al., 2001), mouse (Crep, 1974), cat (Mascitti and Ortega, 1966; Ryu, 1980), rhesus monkey (Bassett et al., 1992), and human (Crosby and Humphrey, 1941; Ohm et al., 1991).

According to Crosby and Humphrey (1939a) the most rostral subdivision of the northern short-tailed shrew (Blarina brevicauda) AON, the pars lateralis, appears as a mass of densely arranged cells within the inner regions of the granule cell layer of the MOB, lateral to the olfactory ventricle. The second subdivision to appear is the pars externa which is separated from the pars lateralis by a fiber mass. Moving caudalward, the middle region of the pars externa disappears and only the dorsal and ventral limbs of
the pars externa remain present. At this level, the pars lateralis begins to change shape
forming a crescent-shaped structure with the concave side open to the olfactory ventricle.
The crescent-shaped region of AON is subdivided into the pars dorsalis, pars lateralis, and pars ventralis. A large portion of MOB remains on the ventromedial surface of each hemisphere at this caudal level. The tips of the crescent-shaped AON begin to turn inward toward each other and merge, forming a ring around the olfactory ventricle. The region where the tips merge forms the pars medialis subdivision. At this level, the rostral piriform cortex also is present lateral to the AON. As the piriform cortex becomes well-developed, the pars lateralis disappears, the pars dorsalis merges with the frontal cortex, and the pars medialis is replaced by the septal area and the nucleus accumbens. The pars ventralis which is dorsal to the olfactory tubercle is gradually replaced by the caudate.
The AON of the mole (Crosby and Humphrey, 1939a) is comparable to the shrew except that the pars posterior is the caudal continuation of the pars ventralis and is located between the caudate nucleus and the polymorph layer of the olfactory tubercle. Crosby and Humphrey (1939a) found no pars posterior in the short-tailed shrews examined.

The northern short-tailed shrews (*Blarina brevicauda*) and the southern short-tailed shrews (*B. carolinensis*) examined in this project had an AON structure similar to the mole as reported by Crosby and Humphrey (1939a). The AON subdivisions in *Blarina* included the pars lateralis, externa, dorsalis, ventralis, medialis, and posterior.

6.2 Results

6.2.1 Cresyl Violet Rostral to Caudal Series

The pars lateralis (PL), the most rostral subdivision of the AON was found deep within the granule cell layer in the MOB (Fig. 64) and was rostral to the accessory olfactory
bulb (AOB). The PL consisted of mass of cells lateral to the olfactory ventricle and subventricular zone and was separated from the surrounding granule cell layer by a region of neuropil. Moving in a caudalward direction, the pars externa (PE) subdivision (Fig. 65) was just ventral to the rostral part of the AOB and dorsolateral to the PL. Continuing caudalward to the level of the caudal part of the AOB, the mid-region of the crescent-shaped PE (Fig. 66) disappeared creating dorsal and ventral limbs. The dorsal limb was immediately ventral to the AOB. The ventral limb was significantly larger than the dorsal limb and was dorsolateral to the PL. The dorsolateral MOB layers began to disappear at this more caudal level. The olfactory ventricle remained present and was surrounded by the subventricular zone. Caudalward, only a small portion of the ventral pars externa remained and was located just dorsal to the MOB granule cell layer. The MOB was crescent-shaped at this caudal level and the MOB layers continued to disappear on the dorsolateral side. The caudal PL was crescent-shaped (Fig. 67) with the concave side facing the olfactory ventricle. At this level, the PL could be divided into the pars dorsalis (PD), PL, and pars ventralis (PV). The PL continued to change its shape at a more caudal level, changing from the crescent shape to a ring-like structure (Fig. 68) that surrounded the olfactory ventricle. The tips of the crescent merged to become the pars medialis (PM) subdivision. The PM was dorsolateral to the remaining crescent-shaped caudal MOB. The rostral piriform cortex was lateral to the AON at this level. The ventral-most remnant of the PE was dorsal to the granule cell layer. At a more caudal level, the PD began to merge with the frontal cortex (Fig. 69). At this level, the ring-like structure of the AON completely encircled the olfactory ventricle. The caudal-most MOB remained crescent-shaped at this level. At the caudal level where the pars
Fig. 64. Light micrograph of the rostral pars lateralis of the anterior olfactory nucleus. The pars lateralis (PL) begins dorsal and lateral to the olfactory ventricle (arrow), deep within granule cell layer (GrL) of the MOB. The frontal cortex (FC) is dorsal to the MOB. The medial surface of the MOB is to the left. 40 µm frozen section, coronal plane, cresyl-violet stain. 5 mm = 250 µm.

Fig. 65. Light micrograph of the rostral pars externa of the anterior olfactory nucleus. The pars externa (PE) appears at the level of the accessory olfactory bulb (arrowhead). Pars lateralis (PL), olfactory ventricle (arrow), frontal cortex (FC). 40 µm frozen section, coronal plane, cresyl-violet stain. 5 mm = 250 µm.
Fig. 66. Light micrograph of the mid-caudal pars externa. The mid-region of the crescent-shaped PE disappears and is subdivided into a dorsal (curved arrow) and ventral limb (PE), pars lateralis (PL), olfactory ventricle (arrow), accessory olfactory bulb (arrowhead), frontal cortex (FC). 40 μm frozen section, coronal plane, cresyl-violet stain. 5 mm = 250 μm.

Fig. 67. Light micrograph of the rostral pars dorsalis and pars ventralis. Pars lateralis (PL) is divided into pars dorsalis (PD) and pars ventralis (PV). Subventricular zone surrounds the olfactory ventricle (arrow), pars externa (PE), frontal cortex (FC). 40 μm frozen section, coronal plane, cresyl-violet stain. 5 mm = 250 μm.
Fig. 68. Light micrograph of the rostral pars medialis. The caudal AON is subdivided into the pars dorsalis (PD), pars medialis (PM), pars ventralis (PV), and PL. Frontal cortex (FC), piriform cortex (PC), ventral pars externa (arrow). 40 µm frozen section, coronal plane, cresyl-violet stain. 5 mm = 250 µm.

Fig. 69. Light micrograph of the mid-caudal pars medialis. The pars medialis (PM) is located between the olfactory ventricle and the MOB granule cell layer. The caudal PD is continuous with the frontal cortex (FC). The piriform cortex (PC) is dorsal to the pars ventralis (PV). Pars Lateralis (PL). 40 µm frozen section, coronal plane, cresyl-violet stain. 5 mm = 250 µm.
dorsalis was continuous with the frontal cortex, the PM and PV (Fig. 70) were
dorsolateral to the thin crescent-shaped MOB. The rostral lateral ventricle was occluded.
The PM and PV merged and formed the pars posterior (PP) (see Fig. 17). This most
caudal AON subdivision was dorsolateral to the caudal-most part of the MOB and ventral
to the lateral ventricle. The trilaminar piriform cortex was well-developed at this level.
Moving caudalward, the PP remained ventral to the lateral ventricle. The piriform cortex
was well-developed and distinct. At the caudal level where the MOB completely
disappeared, the PP (Fig. 71) was ventral to the open lateral ventral and dorsal to the
rostral olfactory tubercle.

6.2.2 Light Microscopy of Pars Lateralis (PL)
The PL (Fig. 72) was located deep within the MOB, dorsolateral to the olfactory ventricle
and its surrounding subventricular zone. The olfactory ventricle was lined with flattened
ependymal cells (Fig. 73). Numerous cilia extended into the open ventricle. A region of
small myelinated axons separated the PL from the olfactory ventricle and subventricular
zone of the MOB. The pyramidal cells (Fig. 74) of the PL had spherical euchromatic
nuclei with distinct nucleoli. These cells measured up to 12 μm in diameter. A
distinctive apical dendrite extended from the pyramidal cells in a dorsolateral direction
toward the PE. Basal dendrites extended radially into the surrounding neuropil of the PL.
The neuropil between the PL and PE consisted of numerous pale dendrites and small
myelinated axons. In the region of the neuropil closest to the PL, the dendrites were
larger in diameter and longer in profile. In the neuropil region closest to the PE, the
dendrites were smaller in diameter and shorter in profile.
Fig. 70. Light micrograph of the caudal pars medialis. The caudal pars medialis (PM) and caudal pars ventralis (PV) remain ventral to the occluded lateral ventricle (arrow). Piriform cortex (PC), frontal cortex (FC). 40 μm frozen section, coronal plane, cresyl-violet stain. 5 mm = 250 μm.

Fig. 71. Light micrograph of the pars posterior. The caudal-most pars posterior (PP) is ventral to the caudate-putamen (curved arrow) and dorsal to the olfactory tubercle (OT). Piriform cortex (PC), corpus callosum (CC), lateral ventricle (arrow). 40 μm frozen section, coronal plane, cresyl-violet stain. 5 mm = 250 μm.
Fig. 72. Light micrograph of the olfactory ventricle and subventricular zone. The pars lateralis (PL) is dorsolateral to the olfactory ventricle (OV) and subventricular zone (SVZ). Clusters of myelinated axons (arrow) separate the PL from the ventricular region. PL dendrites (arrowhead) extend away from the olfactory ventricle. 1 μm epoxy resin section, Richardson stain. 5 mm = 33 μm.

Fig. 73. Light micrograph of ependymal cells. The olfactory ventricle is lined with ependymal cells (arrow) with distinct nucleoli. Cilia (arrowhead) extend into the open lumen. Myelinated axons (curved) in the surrounding neuropil. 1 μm epoxy resin section, Richardson stain. 5 mm = 5 μm.
6.2.3 Light Microscopy of Pars Externa (PE)

The PE consisted of neurons and an adjacent neuropil. The neurons of the PE had euchromatic nuclei with a distinct nucleolus. These cells measured up to 9 μm in diameter. The PE neurons differed from the pyramidal cells of the PL. The PE neurons had a thin layer of perinuclear cytoplasm. Dendrites extending from the PE neurons were not commonly observed. The dorsal limb of the PE (Fig. 75) was ventral to the AOB and the internal plexiform layer and lateral olfactory tract. The ventral limb was adjacent to the granule cell layer of the MOB. The neuropil (Fig. 76) of the PE consisted of myelinated axons, dendrites, capillaries, and several oligodendrocytes.

6.2.4 Light Microscopy of Pars Dorsalis (PD), Pars Medialis (PM), Pars Ventralis (PV), Pars Posterior (PP)

The PD (Fig. 77) consisted of pale-staining neurons and a surround neuropil. The neurons had spherical nuclei with a distinct nucleolus. These neurons were similar to the pyramidal cells of the PL in size and morphology but often lacked apical dendrites. Occasional basal dendrites extended radially from the PD neurons. The neuropil contained dendrites and few myelinated axons.

The PM (Fig. 78) contained pyramidal cells with spherical, euchromatic nuclei. A distinct nucleolus was common. The pyramidal cells of the PM were similar to the pyramidal cells of the PL. Apical dendrites extended dorsomedial from the olfactory ventricle towards the granule cell layer of the MOB. Basal dendrites were radially oriented. The surrounding neuropil consisted of numerous dendrites and small myelinated axons.
Fig. 74. Light micrograph of pyramidal cells of the pars lateralis. Pyramidal cell apical dendrites (arrows) extend to the pars externa. Basal dendrites (arrowhead) extend radially in the neuropil. 1 μm epoxy resin section, Richardson stain. 5 mm = 5 μm.

Fig. 75. Light micrograph of the dorsal limb of the pars externa. The dorsal pars externa (PE) is ventral to the accessory olfactory bulb (AOB). Internal plexiform layer of the AOB/lateral olfactory tract (arrowhead). 1 μm epoxy resin section, Richardson stain. 5 mm = 12 μm.
Fig. 76. Light micrograph of the ventral limb of the pars externa. The ventral pars externa (PE) is surrounded by a neuropil of dendrites (arrow) and small myelinated axons (arrowhead). 1 μm epoxy resin section, Richardson stain. 5 mm = 5 μm.

Fig. 77. Light micrograph of pyramidal cells of the pars dorsalis. Basal dendrites (arrow) extend radially with no specific orientation. The neuropil consists of dendrites (arrowhead) and myelinated axons (curved arrow). 1 μm epoxy resin section, Richardson stain. 5 mm = 4 μm.
The PV (Fig. 79) was comparable to the PM. The pyramidal cells had euchromatic nucleoli and were similar to the pyramidal cells of the PL. The apical dendrite extended dorsomedial from the olfactory ventricle towards the granule cell layer of the MOB. Basal dendrites extended radially from these pyramidal cells. The neuropil consisted of pale-staining dendrites and small myelinated axons.

The pyramidal cells of the PP (Fig. 80) were similar to the pyramidal cells of the PL, PM, and PV in size and morphology. The PP pyramidal cells typically had a euchromatic nucleus with a distinct nucleolus. The apical dendrite extended ventromedially towards the remaining crescent-shaped granule cell layer of the MOB. Basal dendrites extended radially and lacked orientation. The neuropil consisted of pale-staining dendrites and small myelinated axons.

6.2.5 Transmission Electron Microscopy of PL

The pyramidal cells of the PL (Fig. 81) had a spherical, euchromatic nucleus with a narrow rim of cytoplasm. Ribosomes and mitochondria were common. Apical dendrites extended from these pyramidal cells. Axo-somatic synapses were observed on the pyramidal cells. The neuropil of the PL (Fig. 82) consisted of myelinated and unmyelinated axons, dendrites and synaptic junctions. Axo-dendritic asymmetrical synapses were commonly observed. The axon terminal was densely packed with small spherical electron-lucent vesicles. The asymmetrical membrane thickening was characterized by electron density within the postsynaptic region.
Fig. 78. Light micrograph of pyramidal cells of the pars medialis. Apical dendrites (arrows) extend dorsomedially to the granule cell layer of the MOB. Basal dendrite (arrowhead). Small myelinated axon (curved arrow) in the neuropil. 1 μm epoxy resin section, Richardson stain. 5 mm = 4 μm.

Fig. 79. Light micrograph of pyramidal cells of the pars ventralis. Apical dendrites (arrow) extend dorsomedially to the MOB granule cell layer. Basal dendrites (arrowheads). Small myelinated axons (curved arrow). 1 μm epoxy resin section, Richardson stain. 5 mm = 5 μm.
Fig. 80. Light micrograph of pyramidal cells of the pars posterior. Apical dendrites (arrows) extend dorsomedially to the granule cell layer of the MOB. Basal dendrites (arrowheads). Small myelinated axons (curved arrow). 1 μm epoxy resin section, Richardson stain. 5 mm = 5 μm.

Fig. 81. TEM micrograph of pars lateralis pyramidal cell with apical dendrite (arrow). Small myelinated axons (curved arrow) and dendrites (arrowhead) in the surrounding neuropil. 5 mm = 1 μm.
6.2.6 Transmission Electron Microscopy of PE

The PE pyramidal cells (Fig. 83) typically had euchromatic nuclei. A well-developed Golgi was adjacent to the nucleus. These neurons had just a narrow rim of cytoplasm. Ribosomes were organized into rosettes and were evenly distributed throughout the cytoplasm. Numerous mitochondria were common. Axo-somatic synapses were identified. The surrounding neuropil (Fig. 84) contained myelinated and unmyelinated axons and electron-lucent dendrites. Asymmetrical axo-dendritic synapses consisted of a postsynaptic membrane thickening and clusters of spherical vesicles within the presynaptic axon terminal.

6.2.7 Transmission Electron Microscopy of PD, PM, PV, PP

The pyramidal cells of the PD (Fig. 85) had a euchromatic nucleus surrounded by a thin rim of cytoplasm. A well-developed Golgi was adjacent to the nucleus. Rosettes of ribosomes and numerous mitochondria were common. Axo-somatic asymmetrical synapses were common. The neuropil (Fig. 85) of the PD consisted of myelinated and unmyelinated axons and dendrites. Axo-dendritic asymmetrical synapses were commonly observed. The terminal region was densely packed with small electron-lucent spherical vesicles.

The PM, PV, and PP consisted of pyramidal cells similar to the pyramidal cells of the PL (see Fig. 81). The spherical, euchromatic nucleus was surrounded by a thin layer of cytoplasm densely populated with ribosomes and mitochondria. Apical dendrites extended from these pyramidal cells. Axo-somatic synapses were common. The neuropil of the PM, PV, and PP was similar to the neuropil of the PL and consisted of myelinated and unmyelinated axons, dendrites and synaptic junctions. Axo-dendritic
Fig. 82. TEM micrograph of synapses in the pars lateralis neuropil. Axo-dendritic asymmetrical synapses (arrowheads). Spherical vesicles (arrow) in the terminal region. 

5 mm = 0.4 μm.

Fig. 83. TEM micrograph of pars externa pyramidal cell. Small myelinated axon (arrow), axo-somatic synapse (curved arrow). 5 mm = 0.6 μm.
Fig. 84. TEM micrograph of synapses in the pars externa neuropil. Asymmetrical synapses consist of postsynaptic density (arrows) and clusters of electron-lucent vesicles in the presynaptic axon terminal (arrowhead). 5 mm = 0.2 μm.

Fig. 85. TEM micrograph of pars dorsalis pyramidal cell. The neuropil consists of small myelinated axons (curved arrow) and unmyelinated axons and dendrites. Asymmetric axo-dendritic synapse with spherical vesicles in the terminal region (arrow). 5 mm = 0.8 μm.
asymmetrical synapses were common. The axon terminals were densely packed with small spherical vesicles.

6.3 Discussion

The AON of *Blarina* had comparable subdivisions to the AON of other mammals (Herrick, 1924; Haberly and Price, 1978b; Valverde et al., 1989) but the rostral-caudal extent of the AON in *Blarina* appeared to be significantly larger. The most rostral AON subdivision of *Blarina* was the PL which appeared deep within the granule cell layer of the MOB, rostral to the AOB. The PL was completely encircled by the well-developed MOB. In the hedgehog (Valverde et al., 1989), opossum (Herrick, 1924), and mole (Crosby and Humphrey, 1939a), the rostral PL also appears deep within the MOB rostral to the AOB. However, in the mouse (Crosby and Humphrey, 1939a) and rat (Burns, 1982), this most rostral AON subdivision first appears more caudally at the level of the AOB and is only partially surrounded by the MOB since the lateral MOB has disappeared. The most caudal AON subdivision in *Blarina* was the PP. The PP appeared at the level of the rostral olfactory tubercle and mid-caudal piriform cortex. The caudal AON of the hedgehog (Valverde et al., 1989), opossum (Herrick, 1924), mole (Crosby and Humphrey, 1939a), mouse (Crosby and Humphrey, 1939a), and rat (Burns, 1982) also appears at the rostral olfactory tubercle and mid-caudal piriform cortex. In these Insectivora (Crosby and Humphrey, 1939a; Valverde et al., 1989) and marsupials (Herrick, 1924), the AON first appears at a significantly more rostral level than in the rat (Burns, 1982) and the MOB is well-developed from the rostral to caudal levels of the AON. In *Blarina*, the rostral appearance of the AON and the well-developed MOB were similar to the observations of Crosby and Humphrey (1939a) and Valverde and
coworkers (1989). Furthermore, the general appearance of the AON in *Blarina* and these Insectivora and marsupials was rotated counter-clockwise dorsolaterally to accommodate the caudal MOB. Since the MOB of the rat and mouse disappeared more rostrally than in these Insectivora and marsupials, rotation of the AON was not observed in the rat and mouse.

The AON participates in numerous afferent and efferent projection pathways. The MOB is a major projection source to the AON. These MOB projections travel via two pathways, the anterior limb of the anterior commissure and the lateral olfactory tract (Mascitti and Ortega, 1966; Bennett, 1968; Turner et al., 1978; Ojima et al., 1984). All subdivisions of the AON except the PE receive direct input from the ipsilateral mitral and tufted cell axons and collaterals of the MOB. All subdivisions of the AON receive indirect input from the contralateral MOB (Mascitti and Ortega, 1966; Scalia and Winans, 1975; Davis et al., 1978; De Olmos et al., 1978; Meyer, 1981; Schneider and Scott, 1983; Ojima et al., 1984; Shipley and Adamek, 1984; Scott et al., 1985; Bayer, 1986; Brown and Brunjes, 1990; Nickell and Shipley, 1992; McLean and Shipley, 1992; Jansen et al., 1998; Barbado et al., 2001). In additional to the MOB projections, the AON receives projections from the ipsilateral and contralateral AON, the piriform cortex, and the olfactory tubercle (Haberly and Price, 1978b; Luskin and Price, 1983b; Schwob and Price, 1984; Barbado et al., 2001). The MOB receives bilateral AON projections to the contralateral MOB via the anterior commissure and to the ipsilateral MOB (Broadwell, 1975b; MacLeod, 1977; Shafa and Meisami, 1977; De Olmos et al., 1978; Mori et al., 1979; Davis and Macrides, 1981; Macrides et al., 1981; Luskin and Price, 1983a; Alheid et al., 1984; Carson, 1984; Schwob and Price, 1984; Bayer, 1986; McLean and Shipley,
1992; Reyher et al., 1988; Nickell and Shipley, 1992). The AON does not project to the AOB (McLean and Shipley, 1992). According to Haberly and Price (1978b), the extensive projections of the AON serve to coordinate olfactory information on both sides of the brain.

6.3.1 Pars Lateralis (PL) and Subdivisions

The PL was the most rostral subdivision in *Blarina*. In other macrosmatic mammals including the mole (Crosby and Humphrey, 1939a), hedgehog (Valverde et al., 1989), and rat (Haberly and Price, 1978b; Burns, 1982; Brown and Brunjes, 1990), the PL also is the most rostral subdivision and consists of a mass of pyramidal neurons located lateral to the olfactory ventricle. The olfactory ventricle is a rostral extension of the lateral ventricle is lined with ependymal cells that are ultrastructurally similar to ependymal cells lining the ventricles of the brain and the central canal of the spinal cord (Brightman and Palay, 1963; Allen and Low, 1973; Bruni et al, 1973; Scott et al., 1974; Hannah and Geber, 1977; Kaplan, 1980; Masuzawa et al., 1981; Bruni et al., 1985; Bruni and Reddy, 1987). In the rat (Garcia-Ojeda et al., 1998), the apical dendrites of the PL pyramidal cells extend dorsolaterally from the olfactory ventricle towards the PE. In *Blarina*, the distinct apical dendrites also extend dorsolaterally from the olfactory ventricle towards the PE. In other mammals, the PL is not the most rostral subdivision to appear. In the giant panda (Lauer, 1949) and the cat (Kinney, 1982), the pars dorsalis appears as the most rostral subdivision. Moving in a caudalward direction, in *Blarina*, the PL changed from a nearly linear layer of pyramidal cells into a crescent shape consisting of the PD, PL, and PV subdivisions followed by the formation of a ring-like structure surrounding the olfactory ventricle. At this level, the PM was lateral to the olfactory ventricle. This
pattern of the caudal PL, PD, PV, and PM is also observed in the mole (Crosby and Humphrey, 1939a), hedgehog (Valverde et al., 1989), opossum (Herrick, 1924) and rat (Haberly and Price, 1978b). In *Blarina*, the PD was continuous with the frontal cortex which is also observed in numerous mammals (Herrick, 1924; Crosby and Humphrey, 1939a; Romfh, 1982; Valverde et al., 1989; Brown and Brunjes, 1990). In the mouse and mole (Crosby and Humphrey, 1939a), the PM and PV merge to form the most caudal subdivision, the PP. In *Blarina*, the PM and PV also merge and form the PP. In the rat (Brown and Brunjes, 1990) the PP is the caudal continuation of the PV. The pyramidal cells of the caudal subdivisions PM, PV, and PP extend apical dendrites oriented dorsomedially towards the tenia tecta. Basal dendrites extend from the cell body without any specific orientation. The pyramidal cell axons extend towards the olfactory ventricle and anterior commissure (Herrick, 1924, Haberly and Price, 1978b). In *Blarina* the apical dendrites of these caudal subdivisions also extend ventromedially away from the olfactory ventricle towards the crescent-shaped caudal MOB.

Mascitti and Ortega (1966) report axo-somatic and axo-dendritic synapses on the neurons of the PL. In *Blarina*, asymmetrical axo-somatic and axo-dendritic synapses were also identified. Electron-lucent spherical vesicles in the axon terminal and increased electron density in the postsynaptic region were commonly observed. These asymmetrical synapses were similar to the asymmetrical synapses observed in the MOB.

**6.3.2 Pars Externa (PE)**

The PE, due to its position and neurons, is the only AON subdivision that is noticeably distinguished from the other AON subdivisions (Broadwell, 1975b; Barbado et al., 2001).
The PE is ventral to the AOB and is in close proximity to the granule cell layer of the MOB (Young, 1934; MacLeod, 1977; Davis and Macrides, 1981; Bayer, 1986; Reyher et al., 1988). In Blarina, the PE was similar in shape and location to the PE of other mammals. In the rat (Burns, 1982), the mole and shrew (Crosby and Humphrey, 1939a), and the hedgehog (Valverde et al., 1989) the PE is the second AON subdivision to appear. In Blarina, the PE also was the second subdivision to appear. In the shrew and the mole (Crosby and Humphrey, 1939a) and in the opossum (Herrick, 1924), moving in a caudalward direction, the middle region of the PE disappears, leaving only a dorsal and ventral limb of the PE. This pattern was followed in Blarina. The dorsal limb of the PE was located immediately ventral to the AOB and the ventral limb was dorsal to the granule cell layer of the MOB. In the rat (Haberly and Price, 1978b; Reyher et al., 1988), the neurons of the PE differ from the neurons of the PL, PD, PM, PV, and PP. The PE neurons lack basal dendrites. The apical dendrites of these neurons extend away from the olfactory ventricle. Similarly, in the opossum (Herrick, 1924), the PE dendrites extend away from the MOB and penetrate the GrL of the MOB. Axons of the PE neurons extend toward the anterior commissure. In Blarina, by light microscopy, apical dendrites of the PE neurons were not evident.

The PE is reported to be well-developed in macrosmatic mammals with axo-somatic and axo-dendritic synapses (Lauer, 1949; Mascitti and Ortega, 1966). In Blarina, similar axo-somatic and axo-dendritic synapses were commonly identified in the PE. Both types of synapses were characterized as asymmetrical synapses and contained predominantly electron-lucent spherical vesicles and increased electron density in the membrane thickening of the postsynaptic region.
The PE provides a pathway of communication between the two MOBs within macrosmatic mammals (Schoenfeld and Macrides, 1984; Reyher, 1988). The PE receives ipsilateral and contralateral input from MOB mitral and tufted cell axons of the lateral olfactory tract and contralateral input from most subdivisions of the AON (Schoenfeld and Macrides, 1984; Barbado et al., 2001). The PE projects extensively to the contralateral MOB and terminates within the granule cell layer. This subdivision does not appear to project to any other AON subdivisions or to the piriform cortex. A lesion to MOB and AON results in contralateral degeneration in the PE (Herrick, 1924; Mascitti and Ortega, 1966; Shafa and Meisami, 1977; Davis and Macrides, 1981; Luskin and Price, 1983a; Alheid et al., 1984; Carson, 1984; Schoenfeld and Macrides, 1984; Bayer, 1986; Reyher et al., 1988; De Carlos et al., 1989; Brown and Brunjes, 1990; Nickell and Shipley, 1992). No PE has been identified in the adult human or in the macaque which may contribute to a poorly-developed sense of olfaction in these microsmatic mammals (Crosby and Humphrey, 1941; Lauer, 1945).

6.4 Comparative Studies

To compare the AON of various mammals, two methods have been used-percentages and size indices. In this second method, the volume of the AON is plotted against the body weight in double logarithmic scales (Baron et al., 1987; Stephan et al., 1991). Baron and coworkers (1987) report that the AON (retrobulbar region) index in *Blarina brevicauda* had a value of 111. *Sorex fumeus*, the smoky shrew, with an AON index of 115 is comparable to *Blarina*. Other shrews including *Sorex araneus* (93), *Suncus etruscus* (73), and *Neomys fodiens* (81) have smaller AON indices than *Blarina*. Thus it appears that based upon the size index, *Blarina* has a larger AON than most shrews. Two
insectivores that are closely related to *Blarina*, the European hedgehog, *Erinaceus europaeus*, and the European mole, *Talpa europaea*, have an index of 116 and 87 respectively. Comparing terrestrial and semiaquatic shrews, the AON index in terrestrial insectivores is significantly higher (108) than in the semiaquatic insectivores (64). Based upon these indices, the terrestrial shrews, which include *Blarina*, have a well-developed AON that greatly assists these macrosmatic mammals. In addition, the AON index in *Blarina* is significantly higher than in microsmatic mammals such as the macaque (8) and human (18). These low indices appear to indicate a poorly-developed AON. Baron and coworkers (1987) also report percentages of the olfactory cortex which includes the retrobulbar region (AON), prepiriform cortex, and olfactory tubercle. In the Insectivora, the AON makes up approximately 16% of the olfactory cortex. This percentage is higher than the prosimians, simians, and humans which are reported 12%, 5%, and 4% respectively. Therefore, based upon percentages, it appears that the Insectivora compared to the primates have a larger, well-developed AON that would provide more extensive processing of olfactory inputs.

6.5 Conclusion

The AON in *Blarina* was well-developed and had comparable subdivisions identified in other macrosmatic mammals. The AON first appears at a significantly more rostral level and the MOB continues more caudally in *Blarina* than in other mammals. This caudal continuation of the MOB results in a general counter-clockwise dorsolateral rotation of the AON to accommodate the MOB. This rotation of the AON is not observed in the rat or mouse. Based upon the rostral-caudal extend of the AON and MOB and the
morphological observations of the AON, it appears that the AON of *Blarina* is a well-developed olfactory system structure that differs from other small mammals.
CHAPTER VII

OLFACTORY TUBERCLE

7.1 Introduction

The olfactory tubercle (OT) is a trilaminar structure consisting of a superficial plexiform layer, a middle pyramidal layer, and a deep polymorphic layer which has also been called the parvicellular layer (Crosby and Humphrey, 1941; Krieger, 1981; Gordon and Krieger, 1983; Talbot et al., 1988a; Krieger and Scott, 1989). Since the deep layer is more frequently referred to as the polymorphic layer, for the purposes of this paper, the deep layer will be termed the polymorph cell layer. The OT of macrosmatic mammals is a prominent swelling located on the ventral surface of the brain caudal to the AON and medial to the LOT (Krieger, 1981; Millhouse and Heimer, 1984; Shipley et al., 1995). The OT has been studied in numerous mammals including the opossum (Meyer, 1981), guinea pig (Johnson, 1957a), shrew opossum (Herrick, 1921), rat (Okada et al., 1977; Fallon et al., 1978; Ribak and Fallon, 1982; Fallon, 1983; Gordon and Krieger, 1983; Krieger et al., 1983; Friedman and Price, 1984; Millhouse and Heimer, 1984;), rabbit (Young, 1934), cat (Meyer and Wahle, 1986; Wahle and Meyer, 1986; Talbot et al., 1988a, b; Berezhnaya et al., 1999), dog (Berezhnaya et al., 1999), and human (Crosby and Humphrey, 1941). The OT is not present in anosmatic mammals (Smith, 1909) and in microsmatic mammals such as humans, the OT is not well-developed.

The northern short-tailed shrew (*Blarina brevicauda*) and the southern short-tailed shrew (*B. carolinensis*) are considered to be macrosmatic mammals based upon MOB, OT, and piriform cortex size, but the OT of *Blarina* has yet to be examined in detail. The purpose of this part of the project was to examine the OT of *Blarina* by light...
microscopy and, by morphological observations, confirm the hypothesis that the OT of
*Blarina* is a well-developed olfactory structure that differs from other small mammals
and thus provide further evidence that these primitive placental mammals are
macromsmatic mammals with substantial olfactory systems at all levels.

7.2 Results

7.2.1 Light Microscopy Overview

In a sagittal plane of section (Fig. 86) the OT was ventral to the MOB and appeared at the
level of the AOB, AON and frontal cortex. At the most rostral level (Fig. 87), the
piriform cortex and lateral olfactory tract were dorsolateral to the OT. The corpus
callosum was dorsal to the open lateral ventricle. At a mid-caudal level (Fig. 88), the
islands of Calleja extended into the polymorph cell layer. The lateral ventricle was
occluded at this mid-caudal level. The caudate-putamen and frontal cortex were well-
developed. At the most caudal level (Fig. 89), the third ventricle was lateral to the lateral
ventricle. The well-developed piriform cortex was dorsolateral to the OT. The caudate-
putamen was dorsal to the OT and distinct. The three distinct layers of the OT (Fig. 90)
consisted of the outer plexiform layer, middle pyramidal cell layer, and the deep
polymorph cell layer. Clusters of granule cells formed the islands of Calleja which were
located within the polymorph cell layer. These islands also extended across the
pyramidal cell layer into the plexiform layer. Large multipolar neurons were present in
the polymorph cell layer.

7.2.2 Light Microscopy of Plexiform Layer

The plexiform layer (Fig. 91) consisted of a neuropil of pale-staining dendrites,
Fig. 86. Light micrograph of a sagittal plane of the olfactory tubercle (OT). The OT is ventral to the main olfactory bulb (MOB). Frontal cortex (FC), AON pars dorsalis (PD), AON pars lateralis (PL), nucleus accumbens (NA), caudate-putamen (C-P).

40 μm frozen section, sagittal plane, cresyl-violet stain. 5 mm = 250 μm.
Fig. 87. Light micrograph of the rostral olfactory tubercle. The OT is ventral and medial to the piriform cortex (PC) and lateral olfactory tract (arrow). Pyramidal cell layer (curved arrow), islands of Calleja (arrowhead), frontal cortex (FC), lateral ventricle (LV). 40 μm frozen section, coronal plane, cresyl-violet stain. 5 mm = 250 μm.

Fig. 88. Light micrograph of the mid-caudal olfactory tubercle. Islands of Calleja (curved arrow) extend into the polymorph cell layer. Lateral ventricle (LV), frontal cortex (FC), caudate-putamen (C-P), piriform cortex (PC), lateral olfactory tract (arrow). 40 μm frozen section, coronal plane, cresyl-violet stain. 5 mm = 250 μm.
Fig. 89. Light micrograph of the caudal olfactory tubercle. The caudal OT did not typically have Islands of Calleja. Lateral ventricle (LV), piriform cortex (PC), lateral olfactory tract (arrow). 40 μm frozen section, coronal plane, cresyl-violet stain.

5 mm = 250 μm.

Fig. 90. Light micrograph of the trilaminar olfactory tubercle. Multipolar neurons (arrow) in the polymorph cell layer (PO), Islands of Calleja (IC), pyramidal cell layer (PY), plexiform layer (PL). 40 μm frozen section, coronal plane, cresyl-violet stain.

5 mm = 45 μm.
capillaries, and few scattered pyramidal cells. The thickness of the plexiform layer varied depending upon the location of the pyramidal cell layer. The thinnest regions measured approximately 115 μm and the thickness regions were approximately 350 μm deep. The average thickness of the plexiform layer was 175 μm.

7.2.3 Light Microscopy of Pyramidal Cell Layer

The pyramidal layer (Fig. 92) consisted of pyramidal cells and a surrounding neuropil. Pyramidal cell nuclei were spherical and euchromatic with a distinct, darkly-stained nucleolus. Pyramidal cell nuclei measured up to 9 μm in diameter. Nissl bodies were common in thin rim of cytoplasm. Apical dendrites extended towards the plexiform layer. Basal dendrites projected to the deep polymorph cell layer. The average thickness of the pyramidal cell layer varied according to the density of pyramidal cells. In some areas, this layer measured only 35 μm in thickness. In areas of greater pyramidal cell density, the thickness was 125 μm. The average thickness of the pyramidal cell layer was approximately 75 μm. The neuropil of the plexiform layer consisted of pale-staining dendrites, axons and glial cells.

7.2.4 Light Microscopy of Polymorph Cell Layer

The polymorph cell layer (Fig. 93) consisted of three types of cells and a neuropil. Pyramidal cells were scattered throughout the polymorph cell layer and were comparable to the pyramidal cells of the pyramidal cell layer. The second cell type was the multipolar neuron. The multipolar cell nuclei measured up to 19 μm in diameter and had pale-staining nuclei with a distinct nucleolus. Nissl bodies were common in the abundant cytoplasm. An apical dendrite extended towards the pyramidal cell layer. Basal
Fig. 91. Light micrograph of the plexiform layer of the olfactory tubercle. The superficial plexiform layer consisted of pale-staining dendrites (arrow) and scattered pyramidal cells (arrowhead). 1 μm epoxy resin section, Richardson stain. 5 mm = 7 μm.

Fig. 92. Light micrograph of the pyramidal cell layer of the olfactory tubercle. Pyramidal cells (arrowhead) are interspersed in a neuropil of pale-staining dendrites (curved arrow). Apical dendrite (arrow), plexiform layer (PL). 1 μm epoxy resin section, Richardson stain. 5 mm = 4 μm.
dendrites extended mostly horizontally and deeper. The third cell type was the granule cells which were typically grouped in clusters forming the islands of Calleja (Fig. 94). These islands commonly were located in the polymorph cell layer but also were found extending into the pyramidal cell and plexiform layers. These granule cells had heterochromatic nuclei with darkly stained nucleoli. Granule cells were typically spherical and granule cell nuclei measured up to 7 μm in diameter. The thin layer of cytoplasm was not visible by light microscopy. On occasion, pyramidal cells were located within the islands of Calleja. The surrounding neuropil of the polymorph cell layer consisted of numerous pale-staining dendrites, small myelinated axons, and glial cells.

7.3 Discussion

Based upon the morphological data in this current study, the trilaminar structure and cell types of the OT in *Blarina* were similar to that of other macrosmatic mammals. However, there were some differences noted in the OT of *Blarina* when compared to other macrosmatic mammals.

7.3.1 Plexiform Layer

In several species, such as the guinea pig (Johnson, 1957a) and the rat (Gordon and Krieger, 1983), the plexiform layer thickness varies in different regions. In *Blarina*, the thickness of the plexiform layer also varied. The neuropil in *Blarina* was consistent with the typical mammalian plexiform layer neuropil and contained numerous apical dendrites. These apical dendrites are reported to be the apical dendrites of pyramidal cells. In addition, MOB axons are reported to terminate on the pyramidal cell dendrites.
Fig. 93. Light micrograph of the polymorph cell layer of the olfactory tubercle. Multipolar neurons with dendrites (arrow) extending toward the pyramidal cell layer. Pyramidal cells (arrowhead), myelinated axons (curved arrow). 1 μm epoxy resin section, Richardson stain. 5 mm = 8 μm.

Fig. 94. Light micrograph of granule cells of the Islands of Calleja. Granule cells with distinct nucleoli (arrow) of the polymorph cell layer. Occasional pyramidal cells (arrowhead) are located within the islands of Calleja. 1 μm epoxy resin section, Richardson stain. 5 mm = 4 μm.

### 7.3.2 Pyramidal Layer

The pyramidal layer typically contains a dense population of closely packed pyramidal cells that are similar to cortical pyramidal cells (Johnson, 1957a; Ribak and Fallon, 1982). The apical dendrite extends into the plexiform layer and the basal dendrites project toward the polymorph cell layer (Shipley and Ennis, 1996). In *Blarina*, pyramidal cell dendrites followed a similar projection pattern. Variations in pyramidal cell diameters are common. In the rat OT, pyramidal cells measure up to 16 µm (Millhouse and Heimer, 1984). In the cat OT, pyramidal cells are significantly larger and measure up to 25 µm (Meyer and Wahle, 1986). In *Blarina*, pyramidal cells were smaller than reports of other macrosmatic mammals and measured up to 9 µm in diameter.

### 7.3.3 Polymorph Cell Layer

The polymorph cell layer is the thickest layer of the trilaminar OT (Johnson, 1957a) and contains several cell types interspersed among dendrites of pyramidal cells (Meyer and Wahle, 1986). The largest cells are the polymorph cells, also referred to as multipolar cells, which measure, in the rat, up to 40 µm in diameter (Krieger 1981; Millhouse and Heimer, 1984). In *Blarina*, the multipolar neurons were significantly smaller than the rat polymorph cells and measured only 19 µm in nuclear diameter. According to Shipley and Ennis (1996), dendrites of the large neurons do not extend preferentially towards the pyramidal cell layer. In *Blarina*, the primary dendrites commonly projected towards the pyramidal cell layer and basal dendrites extended radially. The second cell type is the
granule cell which clusters together to form structures called the islands of Calleja. These islands are typically located within the polymorph cell layer. However, occasionally the islands of Calleja extend into the plexiform layer (Ribak and Fallon, 1982; Fallon, 1983; Millhouse and Heimer, 1984; Talbot et al., 1988a). In Blarina, the islands of Calleja were also observed extending into the plexiform layer. Granule cells measure approximately 8-12 µm in diameter in the rat (Krieger, 1981; Millhouse and Heimer, 1984). In cats and dogs, Berezhnaya and coworkers (1999) identified three types of cell in the islands of Calleja. The most granule cell is small and measures 7-10 µm in diameter. Talbot and coworkers (1988a) also report comparable measurements for small granule cells. An intermediate size granule cell measuring 25-30 µm in diameter and a large granule cell measuring 35-50 µm in diameter are also common. According to Berezhnaya and coworkers (1999), the small granule cells are the most common. The small granule cells cluster together to form the islands of Calleja whereas the intermediate and large granule cells typically are located more to the periphery of the islands. In Blarina, only one population of granule cells was observed. These granule cells measured 7 µm in diameter and were comparable to the rat granule cells (Krieger, 1981) and small granule cells reported by Berezhnaya and coworkers (1999). In addition, Berezhnaya and coworkers (1999) identify a narrow cytoplasm with three or four radial dendrites extending from the granule cells. In Blarina, the thin granule cell cytoplasm was not visible by light microscopy.

7.4 Comparative Studies

Baron and coworkers (1987) reported OT size indices for various mammals. The OT index in Blarina brevicauda was 113. Sorex fumeus, the smoky shrew, had an index of
116 which is comparable to *Blarina*. Other shrews including *Sorex araneus* (103), *Suncus etruscus* (66), and *Neomys fodiens* (82) have smaller OT indices than *Blarina*.

Thus it appears that based upon the size index, *Blarina* has a larger OT than most shrews.

Two Insectivores closely related to *Blarina*, the European hedgehog, *Erinaceus europaeus*, and the European mole, *Talpa europaea*, had a smaller OT index of 77 and 105 respectively. Comparing terrestrial and semiaquatic shrews, the OT index in terrestrial insectivores is significantly higher (107) than in the semiaquatic insectivores (69). Based upon these indices, the terrestrial shrews, which include *Blarina*, have a well-developed OT that probably provides additional processing capacity for olfactory input. In addition, the OT index in *Blarina* is significantly higher than in microsmatic mammals such as the macaque (45). Based upon statistical data, *Blarina* has a more well-developed OT than other macrosmatic Insectivora and microsmatic mammals.

### 7.5 Conclusion

Light microscopic observations of the OT of *Blarina* revealed a well-developed trilaminar structure with the various cell types commonly reported in other mammals. Although in *Blarina* the cells of the OT are smaller than some other macrosmatic mammals, it appears that the OT is highly-developed for processing olfactory input and this level of development supports the importance of olfaction in shrews.
CHAPTER VIII

PIRIFORM CORTEX

8.1 Introduction

The piriform cortex (PC) is a phylogenetically old part of the cerebral cortex (Haberly and Feig, 1983; Haberly and Bower, 1984; Löscher et al., 1998). It is considered to be the primary olfactory cortex because it is the largest olfactory area receiving direct ipsilateral afferent input from the MOB (Stevens, 1969; Davis and Macrides, 1981; Meyer, 1981; Haberly, 1985; Gracey and Scholfield, 1990; Bassett et al., 1992; Kratskin, 1995; Rosin et al., 1999; Stripling and Patneau, 1999; Johnson et al., 2000; Datiche et al., 2001; Haberly, 2001; Zinyuk et al., 2001). The PC is a trilaminar structure consisting of a superficial Layer I, a middle Layer II, and a deep Layer III (DeOlmos et al., 1978; Zinyuk et al., 2001; Best and Wilson, 2003). The principal neuron of the PC is the pyramidal cell (Johnson et al., 2000; Truong et al., 2002).

The PC has been studied in numerous mammalian species including the opossum (Meyer, 1981; Haberly and Feig, 1983; Haberly and Bower, 1984), guinea pig (Gracey and Scholfield, 1990), rat (Haberly and Price, 1978a, b; Luskin and Price, 1983a, b; Friedman and Price, 1984; Curcio et al., 1985; Anders and Johnson, 1990; Rosin et al., 1999; Stripling and Patneau, 1999; Johnson et al., 2000; Datiche et al., 2001; Zinyuk et al., 2001; Best and Wilson, 2003), mouse (Bartolomei and Greer, 1998), hamster (Davis and Macrides, 1981); rabbit (Ojima et al., 1984), cat (Stevens, 1969; Willey et al., 1983), and monkey (Bassett et al., 1992). The purpose of this portion of olfactory system project was to examine by light microscopy the morphology of the PC of Blarina and to test the hypothesis that its PC is a well-developed olfactory system structure that differs
from other small mammals and thus provide additional evidence that these primitive placental mammals are macrosmatic mammals with substantial olfactory systems at all levels.

8.2 Results

8.2.1 Light Microscopy Overview

The most rostral piriform cortex (see Fig. 68) (PC) first appeared at the level of the ring-like structure of the AON that consisted of the pars dorsalis, pars medialis, pars lateralis, and pars ventralis. The caudal-most region of the MOB was ventromedial to the PC. Just caudal to the AON (Fig. 95), the rostral lateral ventricle, corpus callosum, and caudate-putamen were dorsomedial to the PC. The rostral OT was ventral to the PC and the islands of Calleja of the OT were well developed. At the more caudal level (Fig. 96) the rostral third ventricle was distinct. At this level, the PC was a prominent structure on the lateral surface of the brain. At its caudal-most level (Fig. 97), the PC was dorsolateral to the amygdala and ventrolateral to the hippocampus. The PC was a trilaminar structure (Fig. 98) and consisted of outer Layer I, middle Layer II, and deep Layer III.

8.2.2 Light Microscopy of Layer I

Layer I (Fig. 99) is typically subdivided into Layer Ia and Ib. Layer Ia is often further divided into two subdivisions. The most superficial region of Layer Ia, Layer Iα, consisted of numerous small myelinated axons, few dendrites, capillaries, and glial cells. The deep region of Layer Ia was ventral to the layer of myelinated axons. This region consisted of fewer myelinated axons than the superficial region. Dendrites were common in this deep region of Layer Ia. The deepest region of Layer I, Layer Ib, consisted of
Fig. 95. Light micrograph of the rostral piriform cortex. The rostral piriform cortex (PC) is ventrolateral to the rostral lateral ventricle (arrow) and caudate-putamen (C-P). Islands of Calleja (arrowhead) are distinct at the rostral olfactory tubercle. Lateral olfactory tract (curved arrow). 40 μm frozen section, coronal plane, cresyl-violet stain.

5 mm = 250 μm.

Fig. 96. Light micrograph of the mid-caudal piriform cortex. The mid-caudal piriform cortex (PC) at the level of the rostral third ventricle, caudal lateral ventricle (arrow) and caudate-putamen (C-P). 40 μm frozen section, coronal plane, cresyl-violet stain.

5 mm = 250 μm.
Fig. 97. Light micrograph of the caudal piriform cortex. The most caudal piriform cortex (PC) is dorsolateral to the amygdala (AMY). 40 μm frozen section, coronal plane, cresyl-violet stain. 5 mm = 250 μm.

Fig. 98. Light micrograph of the trilaminar piriform cortex. The piriform cortex consists of an outer Layer I (I), middle Layer II (II), and deep Layer III (III). 40 μm frozen section, coronal plane, cresyl-violet stain. 5 mm = 20 μm.
dendrites which were more numerous and larger than dendrites in the other regions of Layer I. Small myelinated axons were common in Layer Ib. The few somata scattered throughout Layers Ia and Ib appeared to be pyramidal cells. The average thickness of Layer Ia and Ib varied from rostral to caudal. At the most rostral level, Layer Ia was approximately 125 μm thick. Moving caudalward, Layer Ia was 250 μm thick. At the most caudal level, Layer Ia was 140 μm thick. At the most rostral level, Layer Ib was approximately 175 μm thick. Moving caudalward, Layer Ib was 200 μm thick and at the most caudal level, Layer Ib measured 125 μm thick.

8.2.3 Light Microscopy of Layer II

Layer II (Fig. 100) consisted of a dense layer of pyramidal cells. These large pale-staining neurons measured up to 14 μm in diameter with a spherical euchromatic nucleus. Darkly stained Nissl bodies were common in the cytoplasm. Pyramidal cell apical dendrites (Fig. 101) extended into Layer Ib and basal dendrites extended towards Layer III. The surrounding neuropil consisted of capillaries, small myelinated axons and numerous pale-staining dendrites. The average thickness of Layer II varied from rostral to caudal levels. At the most rostral level, Layer II measured approximately 100 μm in thickness. Moving caudalward, the thickness increased to 125 μm in thickness. At the most caudal level, Level II was the thinnest and measured about 90 μm thick.

8.2.4 Light Microscopy of Layer III

Layer III consisted of pale-staining dendrites, myelinated axons, and numerous capillaries. Several types of cells were common in Layer III. Pyramidal cells comparable to the pyramidal cells of Layer II were scattered throughout Layer III.
Fig. 99. Light micrograph of Layer I of the piriform cortex. Layer I is subdivided an outer region of myelinated axons (Ax) and dendrites, a middle region (la), and a deep region (lb) with increased concentrations of dendrites. 1 μm epoxy resin section, Richardson stain. 5 mm = 9 μm.

Fig. 100. Light micrograph of Layer II pyramidal cells of the piriform cortex. Apical dendrites (arrowhead) extend toward the superficial Layer I (I). 1 μm epoxy resin section, Richardson stain. 5 mm = 10 μm.
The apical dendrites of Layer III pyramidal cells extended toward Layer I and the basal dendrites extended deep into Layer III. A second cell type, the multipolar neuron (Fig. 102) was common in Layer III. These cells had spherical euchromatic nuclei and measured up to 25 μm in diameter. Darkly stained nucleoli were common. The abundant cytoplasm contained Nissl bodies. Multipolar cell dendrites extended radially throughout Layer III and lacked specific orientation. The surrounding neuropil was densely populated with pale staining dendrites and numerous small myelinated axons. Darkly-stained nuclei which appeared to be oligodendrocytes were common. The thickness of the rostral Layer III measured approximately 400 μm. At caudal levels, the boundaries of Layer III were not possible to distinguish from the surrounding cortical structures; therefore, accurate measurements of the caudal regions of thickness of Layer III were not possible.

8.3 Discussion

The PC is ventral to the rhinal sulcus and extends caudally from the pars lateralis of the AON to the lateral entorhinal cortex and occupies the majority of the ventral surface of the forebrain (Davis and Macrides, 1981b; Friedman and Price, 1984). The PC participates in reciprocal projections with the MOB. Mitral and tufted cell axons of the MOB project to the ipsilateral PC via the LOT (Stevens, 1969; Meyer, 1981; Luskin and Price, 1983b; Willey et al., 1983; Ojima et al., 1984; Gracey and Scholfield, 1990; Shipley and Ennis, 1996; Fukushima et al., 2002).

8.3.1 Layer I

Layer I has been referred to as the molecular layer (Stevens, 1969; Willey et al., 1983) or
Fig. 101. Light micrograph of pyramidal cell dendrites. Apical dendrites of pyramidal cells (arrows) extend towards Layer I. Basal dendrites (arrowhead) extend deep towards Layer III. 1 μm epoxy resin section, Richardson stain. 5 mm = 5 μm.

Fig. 102. Light micrograph of multipolar neurons of the piriform cortex. Multipolar neurons with abundant cytoplasm (M). Pyramidal cell with basal dendrite (arrowhead). Myelinated axons (curved arrow) are common in the neuropil. Oligodendrocyte (arrow). 1 μm epoxy resin section, Richardson stain. 5 mm = 5 μm.
the plexiform layer (Haberly and Feig, 1983; Haberly, 1985; Shipley and Ennis, 1996; Best and Wilson, 2003). Layer I is subdivided into two subdivisions, a superficial Layer Ia and a deep Layer Ib (Haberly and Feig, 1983). Layer Ia is further subdivided into a superficial region, Layer Ia, which consists of myelinated afferent fibers from the MOB that make excitatory synaptic contact within Layer Ia onto the apical dendrites of Layer II and III pyramidal cells (Mascitti and Ortega, 1966; Stevens, 1969; Davis and Macrides, 1981b; Luskin and Price, 1983b; Willey et al., 1983; Haberly and Bower, 1984; Haberly, 1985; Gracey and Scholfield, 1990; Schwerdtfeger et al., 1990; Bartolomei and Greer, 1998; Löscher et al., 1998; Rosin et al., 1999; Stripling and Patneau, 1999; Best and Wilson, 2003). Just deep to Layer Ia is the remaining region of Layer Ia that contains dendrites, MOB axons and axon terminals, and glial cells (Friedman and Price, 1984; Curcio et al., 1985; Anders and Johnson, 1990). The deep region of Layer I, Layer Ib also contains MOB axons and their collaterals and apical dendrites of Layer II and III pyramidal cells. In addition, fibers from the contralateral PC are common in Layer Ib (Haberly and Price, 1978a; Friedman and Price, 1984; Greer, 1991; Rosin et al., 1999; Johnson et al., 2000; Best and Wilson, 2003). In *Blarina*, Layer I was similar to the typical mammalian Layer I of the piriform cortex.

### 8.3.2 Layer II

Layer II consists of a layer of tightly packed pyramidal cells interspersed in a neuropil of dendritic profiles (Haberly, 1985; Shipley and Ennis, 1996). The apical dendrites of pyramidal cells extend into Layers Ia and Ib where they receive input from the MOB (Haberly and Feig, 1983; Haberly and Bower, 1984; Kratskin, 1995; Bartolomei and Greer, 1998; Truong et al., 2002). Pyramidal cell basal dendrites extend into the deeper
Layer III. The pyramidal cell axons and collaterals radiate throughout Layer III and terminate on other pyramidal cells. Axon collaterals are also common in layer Ib and layer II (Haberly, 1985; Shipley and Ennis, 1996). A second cell type, the pyramidal-type cell has also been reported in Layer II. These cells, referred to as semilunar cells, are similar to pyramidal cells but lack basal dendrites. Apical dendrites of semilunar cells extend into Layer I and their axon extends deep into the PC (Haberly, 1985; Shipley and Ennis, 1996). In Blarina, Layer II also consisted of numerous pyramidal cells that clustered together forming a distinct cell layer. The pyramidal cells in Blarina did not appear to have basal dendrites and thus may be semilunar cells. However, by light microscopy, the presence of these pyramidal-type semilunar cells in Blarina can not be confirmed.

8.3.3 Layer III

Several cell types are common in the deep Layer III. Pyramidal cells similar to Layer II pyramidal cells extend a primary dendrite toward Layer I. Basal dendrites extend into Layer III (Davis and Macrides, 1981; Shipley and Ennis, 1996). In Blarina, pyramidal cells of Layer III were also similar to pyramidal cells common in Layer II and the dendrites also extended in a comparable path. A second cell type, the multipolar cell is uniformly scattered throughout Layer III. These cells have numerous dendrites that radiate in all directions from the cell body but are confined to Layer III. The multipolar neurons are reported to receive excitatory synapses from the pyramidal cells and in turn make excitatory synapses onto the dendrites of other PC pyramidal cells (Shipley and Ennis, 1996; Stripling and Patneau, 1999). Large multipolar cells in the opossum range from 15-25 μm in diameter (Haberly and Feig, 1983). In Blarina, these large neurons
were comparable in size to the opossum multipolar neuron. The multipolar dendrites in *Blarina* extended radially and lacked specific orientation. According to Shipley and Ennis (1996), the typical mammalian neuropil of Layer III contains basal dendrites of Layer II pyramidal cells and numerous axons from multipolar cells and Layer II pyramidal cells. These Layer II pyramidal cell axons project in all directions within Layer III and make axo-dendritic synapses onto Layer III pyramidal cells (Haberly and Bower, 1984). In addition, multipolar cell dendrites are common in Layer III (Shipley and Ennis, 1996). The neuropil of Layer III in *Blarina* was similar to the mammalian Layer III neuropil reported in the literature.

8.4 Comparative Studies

Baron and coworkers (1987) reported PC size indices for various mammals. The PC index in *Blarina brevicauda* was 92. *Sorex fumeus*, the smoky shrew, has an index of 109 which is slightly larger than *Blarina*. Other shrews including *Sorex araneus* (91), *Suncus etruscus* (60), and *Neomys fodiens* (56) have smaller PC indices than *Blarina*. Thus it appears that based upon the size index, *Blarina* has a larger PC than most shrews. Two Insectivores closely related to *Blarina*, the European hedgehog, *Erinaceus europaeus*, and the European mole, *Talpa europaea*, had a smaller PC index of 104 and 83 respectively. Comparing terrestrial and semiaquatic shrews, the PC index in terrestrial insectivores is significantly higher (109) than in the semiaquatic Insectivores (61). Based upon these indices, the terrestrial shrews, which include *Blarina*, have a well-developed PC. In addition, the PC index in *Blarina* is significantly higher than in microsmatic mammals such as the macaque (26) and human (65). The statistical data from Baron and coworkers (1987) further supports the hypothesis that *Blarina* has a well-developed PC.
compared to other shrews and other Insectivores including the mole. Furthermore, the macrosmatic *Blarina* has a higher index than various microsmatic mammals.

### 8.5 Conclusion

Light microscopic observations of the PC of *Blarina* revealed a well-developed trilaminar organization with the various cell types similar to that of other macrosmatic mammals. It appears that the PC is a highly-developed structure for processing olfactory input.
CHAPTER IX

CONCLUSION

9.1 Hypothesis

The light microscopic and transmission electron microscopic observations of the ultrastructure of the olfactory system of the northern short-tailed shrew, *Blarina brevicauda* and the southern short-tailed shrew, *B. carolinensis* confirmed the hypothesis that the olfactory system in *Blarina* is well-developed and differs from that of other macrosomatic mammals.

9.2 Conclusions of Olfactory System Structures

9.2.1 Olfactory Epithelium and Mucosa

In the olfactory epithelium, *Blarina* has a light supporting cell which differs from the supporting cells commonly reported in other mammals. The function of this light supporting cell remains unknown at present. However, the numerous mitochondria and large numbers of small vesicles in the light supporting cells supports the hypothesis that this cell could be involved in transporting molecules or ions between the lamina propria and the fluid layer surrounding the olfactory receptor cilia. Furthermore, the average number of cilia per olfactory epithelium receptor cells was higher in *Blarina* than in the mole and hedgehog. Thus, based upon ultrastructure observations, the olfactory epithelium and mucosa of *Blarina* are well-developed.

9.2.2 Main Olfactory Bulb

In the mammalian MOB, a monolayer of mitral cells is located between the external and internal plexiform layers. Although this monolayer is common in macrosomatic
mammals, it is not present in all macrosmatic mammals. In the hedgehog, the mitral cells are more dispersed within the external and internal plexiform layers and do not form a monolayer. In addition, two primitive Monotremes, the duck-billed platypus (*Ornithorhynchus anatinus*) and the echidna (*Tachyglossus aculeatus*) also both lack the monolayer of mitral cells. The absence of a mitral cell monolayer has also been reported in some reptiles and birds. Since moles, shrews, and hedgehogs are members of the order Insectivora, significant variations among the olfactory system structures would appear to be minimal. The absence of a mitral cell layer in the hedgehog represents a significant variation within an order. The functional significance of mitral cells organized in a monolayer versus a generalized displacement remains unclear. It is also unclear why the hedgehog would retain this primitive MOB characteristic and moles and shrews would not retain this feature. Additionally, the caudal-most extent of the MOB of the *Blarina* continued to the level of the piriform cortex. In other macrosmatic mammals such as the rat and mouse, the MOB does not extend as caudally as in *Blarina*. Thus it appears that the MOB of *Blarina* is proportionately larger and extends over a larger brain area than in other macrosmatic mammals such as rodents.

Myelinated dendrites and neuronal somas were observed in the MOB of *Blarina*. Structures similarly have been reported in the squirrel monkey and in the mouse but these appear to be rare observations. In *Blarina*, due to the rarity of these myelinated structures, it appears that these structures probably play no significant role in the olfactory sensory system.

An open olfactory ventricle was common in the MOB of *Blarina*. This feature has also been reported in primitive mammals such as the hedgehog, mole, and opossum.
Open olfactory ventricles have also been reported in several reptiles including the turtle (*Pseudemys elegans*), Gila monster (*Heloderma suspectum*), and the copperhead (*Agkistrodon mokasen*). Occluded olfactory ventricles have been observed in the rat and mouse. In the human fetus, the olfactory ventricle is patent but only rarely does the olfactory ventricle remain in the human adult. Thus it appears that the presence of an open olfactory ventricle is a characteristic of the evolutionary primitive vertebrates.

### 9.2.3 Accessory Olfactory Bulb

The mammalian AOB is reported to be less developed with poorly-defined layers compared to the MOB. The AOB of *Blarina* has a less distinct laminar organization than the complementary MOB layers. In *Blarina*, the AOB mitral cells do not form a distinct monolayer rather the mitral/tufted cells were more dispersed throughout the external plexiform layer so these layers were combined and resulted in the external plexiform/mitral-tufted cell layer. The location of the AOB of *Blarina* was different compared to other macrosmatic mammals. In *Blarina* the AOB appears to develop inside of the MOB adjacent to the external plexiform and granule cell layer of the MOB, not infolded into the MOB as in the mouse and rat nor does the AOB appear as an eminence on the surface of the MOB as in the opossum and rabbit. The *Blarina* AOB size index is smaller than other shrews and other Insectivores, including the mole and hedgehog. Due to the small AOB size index and the poorly-defined layers, it appears that the AOB of *Blarina* is less well-developed compared to other macrosmatic mammals.
9.2.4 Anterior Olfactory Nucleus

The structure and subdivisions of the AON of Blarina were comparable to other macrosmatic mammals, but significant variations in the rostral to caudal extent of the AON in Blarina were noted. In Blarina the AON first appears at a significantly more rostral level than in the rat and the mouse. In Blarina the AON is first observed rostral to the AOB and it continues caudally to the level of the rostral OT. In addition, the MOB of Blarina also continues caudally to the level of the caudal-most AON subdivision, the PP. To accommodate the caudal continuation of the MOB, the AON is rotated counterclockwise and displaced dorsolaterally. This rotation is not observed in the rat or mouse. In Blarina, the shape of the various AON subdivisions was similar to other macrosmatic mammals including the crescent shape and the ring-like structure. Due to the extensive caudal continuation and the ultrastructurally distinct cells of the AON, it appears that this olfactory system structure is well-developed in the macrosmatic Blarina.

9.2.5 Olfactory Tubercle

The OT of Blarina consisted of similar cell types and laminar organization reported in the mammalian OT. However, several differences were noted in Blarina. The pyramidal cells and multipolar cells were smaller than those of more highly evolved mammals such as the rat. Although the layers were thin and the cell types were smaller in Blarina, each layer was well-organized and the various cells were numerous and distinct. Thus it appears that the OT in Blarina is highly-developed for processing important olfactory input for these shrews.
9.2.6 Piriform Cortex

The PC of Blarina had a distinct trilaminar structure similar to the typical mammalian PC. However, several differences were identified. In Blarina, the pyramidal cells were smaller than pyramidal cells of other macrosmatic mammals but the multipolar cells in the shrew were similar in diameter to other mammals. Semilunar cells are reported in the PC of other mammals. These cells resemble pyramidal cells but lack basal dendrites. In Blarina, numerous pyramidal cells with and without basal dendrites were common. By light microscopy, the presence of the semilunar cell can not be confirmed. Light microscopy of the PC revealed a trilaminar structure comparable to other macrosmatic mammals and thus it was concluded that the PC of Blarina was well-developed in these small mammals.

9.3 Summary of the Ultrastructure of the Olfactory System Structures

The physical environment of Blarina may have had an evolutionary impact upon its various sensory systems. The reduction in the visual system appears to have caused the enhancement of and compensation by the olfactory system. Based upon the ultrastructure of the olfactory system structures by light and transmission electron microscopy of the northern short-tailed shrew, Blarina brevicauda and the southern short-tailed shrew, B. carolinensis, it is concluded that the olfactory system of these small mammals differs from other macrosmatic mammals. Furthermore, the large amount of brain volume occupied by the olfactory system and the large surface area of the olfactory epithelium are both consistent with the importance of the olfactory system in these shrews.
CHAPTER X

REFERENCES


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


Goldmakher GV, Moss RL (2000): A subset of periglomerular neurons in the rat accessory olfactory bulb may be excited by GABA through a Na⁺-dependent mechanism. *Brain Res* 871: 7-15.


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


Jesperich MW (1945): The nuclear pattern and the fiber connections of certain non-cortical areas of the telencephalon of the mink (*Mustela vison*). *J Comp Neurol 83*: 173-211.


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


McCotter RE (1912): The connection of the vomeronasal nerves with the accessory olfactory bulb in the opossum and other mammals. *Anat Rec* 6: 299-318.


Okada Y, Hosoya Y, Kurosawa F (1977): High concentration of γ-aminobutyric acid (GABA) and glutamate decarboxylase (GAD) activity in the regions of medial forebrain bundle, diagonal band of Broca and the third layer of the olfactory tubercle. *Proc Japan Acad* 53: 236-240.


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.


186


Treloar HB, Purcell AL, Greer CA (1999): Glomerular formation in the developing rat olfactory bulb. J Comp Neurol 413: 289-304.


LISA JOHNSON BYRUM
Ph.D. Candidate
Department of Biological Sciences
Old Dominion University
Norfolk, Virginia 23529-0276

EDUCATION
2004 Ph.D. Biomedical Sciences, Old Dominion University
1995 M.S. Biological Sciences, Old Dominion University
1986 B.S. Elementary Education, Old Dominion University

RESEARCH INTEREST
Cell ultrastructure, neurobiology, olfactory system

EXPERIENCE
Fall 1996-present Research Assistant, Old Dominion University
Fall 1996 Research Assistant, Diabetes Institute, Eastern Virginia Medical School
1994-1995 Teaching Assistant, Old Dominion University
Spring/Summer 1996 Instructor, Paul D. Camp Community College

PUBLICATIONS


PROFESSIONAL SOCIETIES
Sigma Xi, The Scientific Research Society
America Society of Mammalogists
Virginia Academy of Science